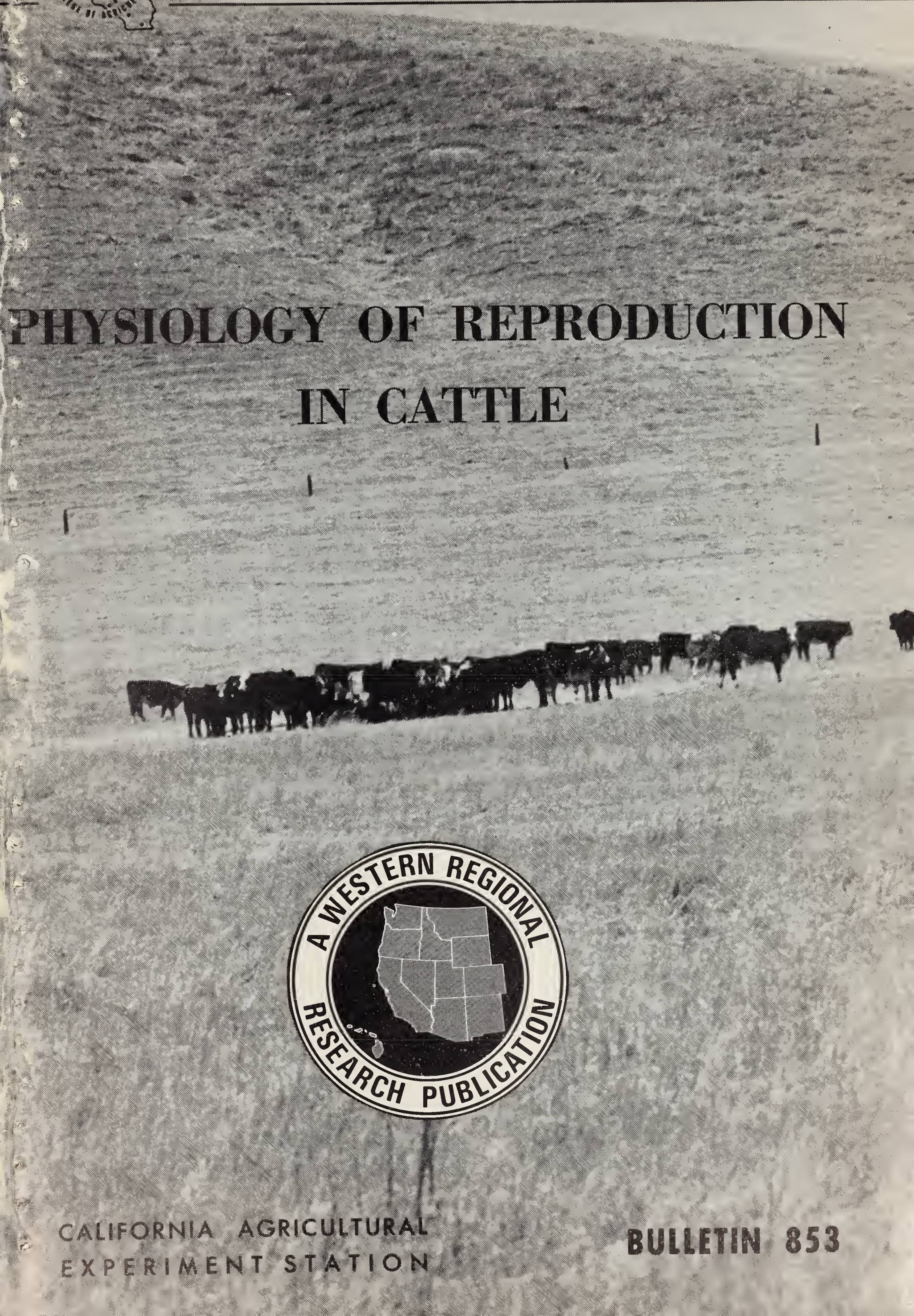
Division of Agricultural Sciences

UNIVERSITY OF CALIFORNIA

PHYSIOLOGY OF REPRODUCTION IN CATTLE



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This bulletin discusses data gathered from western regional research on physiological factors which control normal reproduction in cattle, and assesses changes caused by sterility or lowered reproductive efficiency.

SEPTEMBER, 1971

Names of contributing authors will be found on page 66.

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Under procedure for cooperative publications, this regional report becomes in effect an identical publication of each of the cooperating agencies, and is mailed under the frank and indicia of each.

PHYSIOLOGY OF REPRODUCTION IN CATTLE[1]

INTRODUCTION

Functional infertility is one of the most difficult and costly problems in the livestock industry. The intricate interaction of hormones and organ systems in coordinating and supporting each essential step in normal reproduction provides multiple possibilities for critical mishaps. Undoubtedly, many economically important anomalies of reproduction could be avoided or ameliorated if more were known about their cause and the functional impairment involved.

Western Regional Research Projects W-2, W-49 and W-95 represent three phases of research into the problem. The first (W-2) emphasized nutritional factors influencing reproduction and the improvement of fertility of semen used in artificial insemination. It appeared that nutrition was not a factor if nutrient requirements for growth and production were met. Rapid improvement of techniques for selecting highly fertile bulls whose semen can be used for artificial insemination, and the use of deep frozen fertile bull sperm, tends to reduce the influence of the bull as a cause of infertility. Veneral diseases can be controlled by breeding artificially with semen from healthy bulls, and fertility testing of bulls used naturally (especially under range conditions) has been proved. In the judgment of the Technical Committee our present knowledge of male fertility is adequate for supporting high fertility. Nevertheless, continued study of the hormonal balances in the male is essential for an over-all evaluation of fertility.

The reproductive fitness of the cow population is presently the major limiting factor in making further general improvement in reproductive efficiency. Therefore, the second phase (W-49) of the Western Regional Research was devoted to defining physiological differences associated with normal and abnormal reproduction in cows.

Though it was generally known that the reproductive cycle was controlled by an interplay of hormones, practical methods for measuring the hormones were just being developed and specific hormones and their metabolites of the cow and other domestic animals were only partially known. Many analyses of herd records had been made and these were useful in describing the extent of reproductive abnormalities, but it was obvious that analyses of herd records could only define such problems and not lead to their solution. During this second phase, much fundamental information was gathered regarding tissue and organ changes, identification and metabolism of hormones, development of methods for chemical assay, and measurement of hormones in organs, blood and urine. Work on estrus synchronization was started and attempts were made to reduce the postpartum interval of low fertility.

The third phase (W-95)—represented by the present publication—emphasizes the use of highly sensitive and comparatively rapid analytical methods for measuring changes in the various hormones during the reproductive cycle. Changes associated with normal and abnormal reproduction will be partially defined upon completion of this phase. In this phase there has been increased emphasis on understanding the control of the reproductive cycle in cattle and sheep, the effects of high environmental temperatures on conception rate, and the endocrinology of the postpartum cow. Research is also underway on adrenal in-

[1] Submitted for publication December 29, 1970.

volvement and procedures for producing multiple ovulations in cattle.

The next obvious phase is to apply our fundamental knowledge to the problem of fertility control. Such control includes reduction of time from calving to the first normal estrus, estrus synchronization with high fertility at the synchronized estrus, increased percentages of living calves per 100 cows, and control of time of parturition. Research should be expanded on induction of multiple births in meat-producing cattle. The problem of the retained placenta also will have to be solved to avoid low fertility following twinning.

Of great significance to future problem-solving in the areas mentioned is the increased numbers of well-equipped laboratories, and the increased personnel at the cooperating stations who have specialized training to conduct this type of research in depth. Reaching this stage of development has been painfully slow, mainly because of limited resources, but the committee appreciates the support and interest of the Western Directors. Despite improved techniques and methods in research, however, no one should have illusions about the magnitude and the cost of the future research which will be required to solve the problems involved in increasing reproductive efficiency. Now that a general backlog of fundamental knowledge has accumulated, it probably will be necessary to establish priorities and focus our limited resources on specific problems.

EXPERIMENTAL PROCEDURES

A wide variety of approaches was used by the contributing stations in conducting the research involved in this publication. Some procedures were already available in the literature, others represent modifications of previously used methods, and still others were developed specifically for the research involved. In all the studies care was taken to provide proper control so as to allow for valid comparisons among responses being measured, and appropriate statistical methods were applied where indicated to allow the assigning of probability values.

Although dairy and beef cattle were used primarily in the study, some research was also conducted with sheep and laboratory animals (use of laboratory animals was confined primarily to hormone analysis). Because of the potential influence of genetics on reproduction, specific comparisons were made between beef and dairy cattle, and among various breeds of cattle and their crosses.

Histology

Histological analyses were made, using standard technique procedures for the light microscope. These analyses included the corpus luteum, the adrenal, the endometrium, and the testis. Electron microscopy was also used in studying fine structures of the sperm.

Estrus detection

Estrus was determined by use of teaser animals, heat detector pads, and visual observation. In studies involving intensity of estrus, scoring systems were used.

Ovarian activity

Ovarian structures were identified and the required information was obtained through rectal palpatation and by laparotomy. Function was measured in terms of morphology, histology, and hormone content. Examination of the female reproductive system in addition to the ovaries was made by rectal palpatation and surgery to determine pregnancy, and to measure uterine involution.

Gonadotropin analysis

Pituitary glands were analyzed unextracted or extracted with physiological saline. Venous plasma was extracted by the method described by Anderson and McShan (1966), or serum was analyzed unextracted or extracted with phospate buffer and further separated on a Sephedex G-100 column (Lunnen and W. C. Foote, 1967*).[2] Placental tissue was an-

[2] References with an asterisk indicate publications reporting the results of experiments conducted in this regional research project.

alyzed unextracted or, following extraction, with ammonium sulphate or phosphate buffer solutions (Lunnen and W. C. Foote, 1967*). Placental tissue was also extracted, using ammonium carbonate buffer.

Analyses for LH and LH-like activity were made using the ventral prostate gland response in immature hypophysectomized rats, or the ovarian ascorbic acid depletion method (OAAD, Parlow, 1961), or the radio-immunoassay method (RIA, Niswender *et al.*, 1969). Analyses for FSH were made, using the HCG augmentation method. (Steelman and Pohley, 1953.)

Steroid analysis

An *in vitro* method was developed to determine the potential ability of adrenal glands to synthesize various hormones (Cupps, Laben and Huff, 1969*) and for luteal activity (Sasser and Cupps, 1968*).

Development of chemical methods for hormone assay depends upon identification of the compounds to be measured and the availability of authentic standards in pure form. Over a 15-year period, a workable method has evolved for measuring the principal estrogens in cow urine (Smith, Dickson and Erb, 1956*; Gorski *et al.*, 1957*a,b**; Gorski and Erb, 1959*; Mellin, Erb and Estergreen, 1962*; 1965*; Gomes, Mellin and Erb, 1965*b,c**; Jones and Erb, 1967*; Surve, 1969*; Hunter *et al.*, 1970). Highly sensitive micromethods have been reported for measuring the estrogens in blood, but these are not presently in general use.

Three estrogens, estrone, estradiol-17α and estradiol-17β have been identified in cow placentae (Gorski and Erb, 1959*) and urine (Pope and McNaughton, 1956; Klyne and Wright, 1956; Mellin *et al.*, 1965*; Mellin and Erb, 1966*). Urinary creatinine has been shown to be a satisfactory index substance for expressing rate of excretion of steroids in urine of cows (Indiana), sheep (Plotka and Erb, 1969*) and sows (Erb *et al.*, 1970*), thereby making total collection of urine unnecessary. The correlation between steroid excretion rate, expressed as μg/mg urinary creatinine and as μg/hr based on urine volume, was 0.94 (n = 549) for cows during late pregnancy and after calving (Indiana). Creatinine excretion in the cow increases from 0.94 mg per kg per hour 30 days before calving to 1.09 during labor to 1.19 twelve hours postpartum, and then decreased to 0.83 hours by 40 days postpartum. Use of creatinine as an index compound to express comparative rates of excretion of metabolites of endogenous steroids in urine makes it possible to use larger groups of experimental animals with minimum labor and minimum restraint, thereby reducing anxiety and discomfort. These latter factors may produce artifacts of excretion because of changes in function of the adrenal cortex.

A method for collecting blood from the posterior vena cava was developed at the Montana Station (Hull, 1967*). The posterior vena cava was cannulated about 0.5 cm posterior to the level of the right kidney using Silastic Silicone rubber tubing (Dow Corning Corporation, Midland, Michigan). After collection of the sample, the cannula was flushed with 5 to 10 ml heparin solution (2000 IU per ml normal saline). Heparin solution was injected every day even though no samples were collected. To prevent the growth of fungus or other microorganisms in the heparin solution Terramycin was included in the stock solution (1 mg per ml).

One chemical assay for progesterone and 20β-hydroxy-pregn-4-ene-3-one (20β) in tissues was developed (Gorski *et al.*, 1958a*; Stormshak, Hunt and Erb, 1961*) and used for all such assays reported in this bulletin. Assays for progesterone and 20β in blood were changed as more sensitive techniques were reported by others. Initially, jugular and ovarian venous plasma samples were assayed for progesterone and 20β by the method of Short (1958*a*) as modified by Gomes (1962*) and Gomes *et al.* (1963*). Because of the low quantity of progesterone found in peripheral blood, the double isotope derivative (DID) method of Woolever and Goldfein (1963) was adapted for use (Gomes, Herschler and Erb, 1965*; Erb *et al.*, 1968*a**). Later, simultaneous determination of progesterone and 20β was achieved using gas liquid

chromatography for the quantitative step (Campen, 1969*; Campen and Estergreen, 1969*). At the present time, competitive protein binding developed by Neill *et al.* (1967), has been modified for general use by five of the cooperating stations. In addition to progesterone (Arizona, Colorado, Indiana, Montana, Washington) procedures for measuring cortisol, corticosterone (Indiana), testosterone and estradiol-17β (Colorado) have been developed.

Ultra-violet and DID procedures for measuring progesterone in ovarian venous plasma from cows were in agreement (Erb *et al.*, 1968*a**). Use of competitive protein binding or gas liquid chromatography results in similar values for progesterone in cow peripheral plasma (Washington), but levels determined by DID are approximately one-third higher compared to competitive protein binding (Erb, Randel and Callahan, 1971*). This discrepancy is not understood, as the latter two methods were in excellent agreement when compared using peripheral blood plasma from pregnant and nonpregnant sows (Erb, Randel and Callahan, 1971*; Tillson, Erb and Niswender, 1970*). Though differences in absolute levels of progesterone in cow plasma have been reported, the physiological interpretations based on use of different methods have been the same (Erb *et al.*, 1971*). Presently, there is no satisfactory basis for determining the causes of these discrepancies as one cannot be sure of the completeness of extraction of endogenous progesterone from cow plasma, or the efficiency of removing impurities and of formation of derivatives of progesterone extracted (Erb *et al.*, 1968*a**; 1971*).

Other research was conducted to determine the necessity for measuring progestins in ovarian tissue other than luteal tissue (Erb *et al.*, 1968b*). Progesterone and 20β in the ovary minus its luteal tissue, or in the opposite ovary containing no CL, was only 2.6 per cent of the total. The part-whole linear correlation between progesterone content of the CL and total progesterone in the ovary was 0.99; for ovary progestin content it was 0.96. Thus, the content of progestin in the CL reflects the progestin content of both ovaries. These results are of value for simplifying future research, and they allow more positive interpretation of earlier research in which only progesterone in the CL was measured (Erb *et al.*, 1968*a;* 1971*).

The isolation and identification of progestational compounds other than progesterone from the bovine ovary had not been reported previous to the initiation of this regional project. Early in the project, 20β was isolated from extracts of corpora lutea and ovaries of dairy cattle by Gorski *et al.* (1958*a**) This substance was identified by mobility on paper chromatograms, the formation of derivatives, and by ultraviolet and sulfuric acid spectra. It was found that 20β formed a significant portion of the total progestins extractable from ovarian tissue, so subsequently all assays were done for both progesterone and 20β. Both progestins have been isolated from corpora lutea, residual ovarian tissue, adrenal glands, and ovarian venous plasma (Gorski, *et al.*, 1958*a**; Stormshak and Erb, 1961*, Gomes *et al.*, 1963*). Neither progestin was detected in bovine placentae, uterine venous blood, and the blood, adrenals and testis of a fetus from a heifer pregnant 258 days (Gorski *et al.*, 1958*b**).

Exogenous hormone treatment

A wide variety of hormone treatments involving the whole spectrum of reproductive processes was studied. Hormones included naturally-occurring and synthetic progestins, estrogens, corticosteroids, relaxin, insulin, HCG, PMS, LH and FSH. These hormone preparations were administered in solution intravenously, or in solution or suspension intramuscularly and subcutaneously. Antibodies to ovine LH (anti-LH serum) were produced in rabbits and injected intravenously into sheep to tie-up endogenous circulating levels of LH. Effects of these treatments on reproductive endocrinology were studied in intact cycling, in pregnant animals, and in surgically modified animals.

Surgery and biopsy

The use of standard surgical techniques as well as those requiring modification were employed to provide ovariectomized, adrenalectomized, mastectomized, hysterectomized, thyroidectomized, and parathyroidectomized animals. Observational surgery (laparotomy) was used to provide for collection and for visual observation of tissues and organs, and for their collection including ovaries, oviducts, and blood. Uterine biopsies were obtained by using an intra-cervical approach (Johnson, 1963*).

Environmental control

In addition to environments produced by changes in ambient temperature and humidity, a controlled environmental chamber large enough to hold four cows was constructed to simulate ambient temperatures and humidities. Facilities for confinement of cows under refrigerated conditions were also constructed. Evaporative cooling equipment was used to modify existing shade during high temperature periods (Wiersma and Stott, 1966*).

Measurements of factors contributing to parturition problems

Measurements of pelvic opening, hip width, rump length, and body weight were taken to relate to parturition problems. Analyses of causes of neonatal loss (including postmortem examination) were conducted, and calving difficulty was quantitated by use of a numerical scoring system.

Semen analysis

Chemical analyses of semen samples included citric acid, free amino acids, and fructose or glycerolphosphorylcholine. In addition to microscopic analysis of sperm morphology, analyses were also made of sperm density and motility.

RESULTS AND DISCUSSION

PHYSIOLOGY OF THE FEMALE: PRE-GESTATION

Puberty

Attempts to induce precocious puberty in 10-month-old Hereford heifers were only partially successful. The animals were fed an orally active progestogen (dihydroxyprogesterone acetophenide) and given estradiol valerate injections with or without FSH. Standing estrus was obtained in 11 of 14 heifers, but frequency of ovulation and establishment of estrous cycles were low (U.S.D.A. Miles City).

Estrous cycle

It is now generally agreed that weight, morphology, and progesterone content of the corpus luteum (CL), and concentration of progesterone in ovarian venous and peripheral blood plasma, provides an estimate of luteal function in normal cows during the estrous cycle. The relationships between these measurements are summarized in figure 1 (Erb, Randel, and Callahan, 1971*). The luteal content of progesterone mimics the changes in weight remarkably well, though luteal function declines in 2 days as compared to 6 days for morphological luteal regression. The level of progesterone in ovarian venous plasma agrees rather well with the first and third phases of weight increase of CL and matches the decline in luteal function on day 16 (figure 1, Gomes *et al.*, 1963*). Significant ($P<.01$) correlation coefficients of 0.47 and 0.55 were found when ovarian venous plasma progesterone was compared to luteal content and concentration of progestins, respectively (Gomes *et al.*, 1963*). The growth of the CL as shown in figure 1 may be caused by high titres of estrogen based on excretion of urine (days 6 to 8)

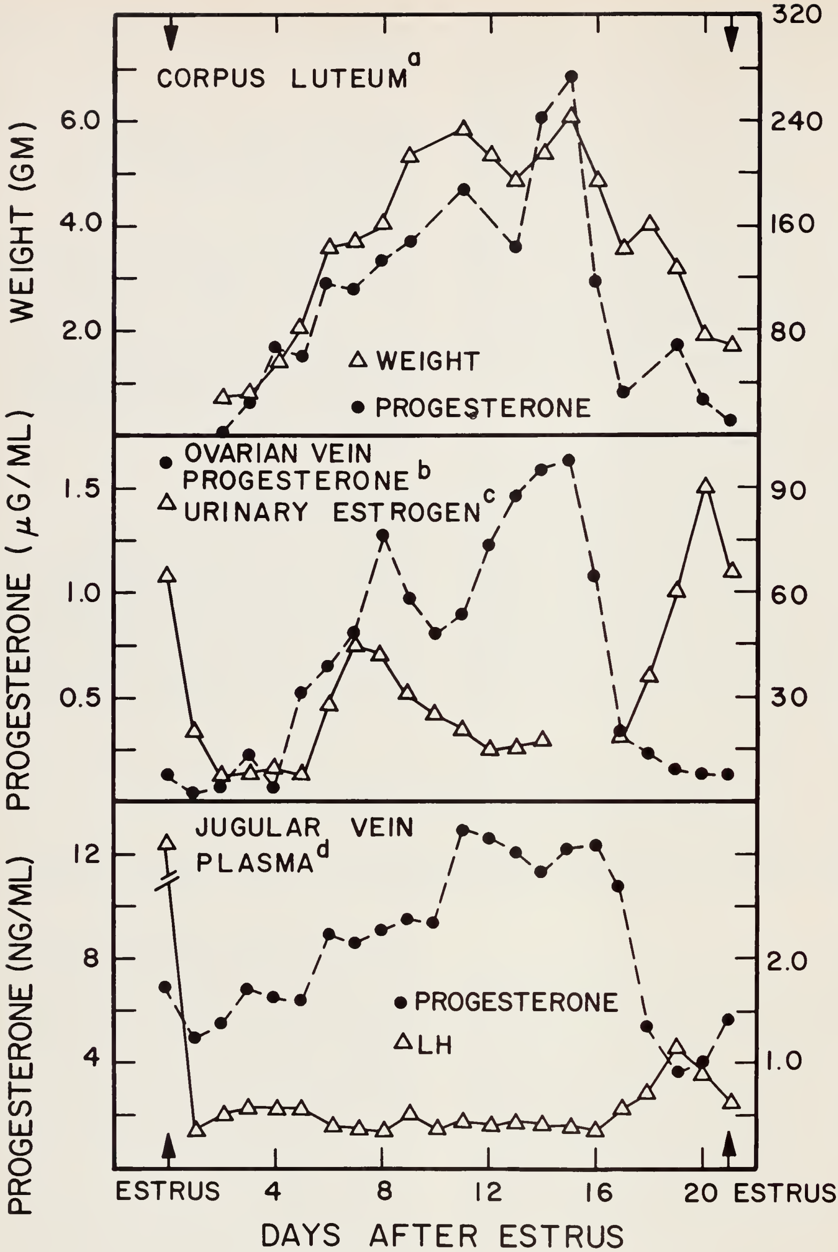


Fig. 1. Comparison of weight of corpus luteum (CL), progesterone in CL, ovarian venous plasma and jugular venous plasma, LH in jugular venous plasma, and estrogen excreted in urine during the estrous cycle of the nonpregnant dairy cow. [a]Foote et al. (1959); Loy, Zimbelman and Casida (1960); Zimbelman, Loy and Casida (1961); Erb and Stormshak (1961*); Mares, Zimbelman and Casida (1962); Gomes et al. (1963*); Foley et al. (1964). N = 200 for CL weight and 147 for CL progesterone; [b]Estimated from Dobrowolski, Stupnicka and Domanski (1968); [c]Redrawn from Mellin and Erb (1966*); [d]Garverick, H. A., R. E. Erb, C. J. Callahan and G. D. Niswender, unpublished data, Purdue University. N = 220 samples from 10 animals.

and high titres of progesterone (days 11 to 13). Preliminary data for total estrogen in blood plasma from the posterior vena cava indicate that the average titres are highest on days 0 to 2 and lowest 3 to 5 days after estrus. There was only minor evidence of increased titres on days 6 to 8 and 15 to 17 (Varman *et al.*, 1964). Diphasic growth of the tertiary follicles also has been described for the cow (Cupps, Laben, and Mead, 1959*; Asdell, 1969; Bane and Rajakoski, 1961). The first phase occurs during days 3 to 6 of the cycle, and the second preceding ovulation.

The preovulatory surge of LH was missed for the data shown in the lower portion of figure 1 because of the sampling schedule. Of interest is the increase in LH starting on day 16 and peaking on day 19 which would have preceded the preovulatory surge by at least 2 days. The correlation between progesterone and LH was –.39 ($P<.05$) during the luteolytic phase. Normally plasma progesterone is low 2 to 4 days before estrus, and excretion of estradiol-17β in urine is significantly higher during the LH rise during proestrus than immediately after the preovulatory surge on the day of estrus.

The relatively slow increase in the levels of progesterone in peripheral plasma, even though luteal function appears to increase rapidly, may be due to uptake of progesterone by mammary tissue (Heap and Linzell, 1966; Chatterton, Chatterton and Hellman, 1969).

Effects of exogenous gonadotropins

Forty-two Hereford heifers were injected with PMS and HCG, bred, palpated per rectum to detect ovulation time, and slaughtered at 3, 30, 45, 60, 75, or 90 days post-ovulation (Hafez, Estergreen and Foster, 1965*). The ovaries were collected and the corpora lutea dissected, weighed, and analyzed for progesterone and 20β. The corpora lutea of singly ovulating animals averaged 50 to 60 per cent heavier than the corpora lutea of animals having multiple ovulation, but the total weight of luteal tissue per cow was 2 to 9 times greater for those having multiple ovulations. The differences in average and total weight of luteal tissue were highly significant ($P<.01$) between those animals having single and multiple ovulations. There was no statistically significant difference in the progesterone concentration in luteal tissue between heifers who ovulated singly and those who had multiple ovulations. Therefore, the differences in the total amount of progesterone between these two groups was due primarily to differences in weight and numbers of corpora lutea. The total amount of luteal progesterone per animal was significantly higher ($P<.01$) in animals with multiple ovulations than in the singly ovulating animal. However, the amount of progesterone per corpus luteum was less in the multiple ovulating animals because their corpora lutea were smaller. On the basis of these data there is little evidence that progesterone would be deficient in multiple pregnancy, although it is possible that too much may be present for the number of surviving embryos.

Effects of exogenous estradiol

The effects of a single injection of 2 mg estradiol-17β on day 14 of the estrous cycle, with and without subsequent daily injections of 0.5 to 2.5 mg estradiol and 5 mg progesterone beginning on day 3, on ovarian function in mature cycling ewes were studied in two different years. Estradiol was administered from day 4 to day 22 of the cycle. Progesterone was administered from day 3 to day 22. Observations were made at various times during and following the hormone treatment period. Tables 1, 2 and 3 show the results. (All tables will be found at end of text, beginning on page 42.) A single injection of estradiol consistently induced CL function. Continued daily injections for 18 days maintained CL function for at least 28 days after termination of treatment as measured by gross examination. Corpora lutea forming on day 6 that were induced with estradiol injected on day 4 followed the same regression pattern as CL naturally forming at the beginning of the cycle. No differences in mean CL weight were found for control ewes at days 10 and 15, or for estradiol

treated ewes at days 10, 15, 22, and 37.

Histological analysis of the CL indicated that they were functional for as long as 48 days following the beginning of estradiol treatment, although some regressive changes were observed. Daily injection of estradiol resulted in decreased follicular activity extending for at least 2 weeks after termination of treatment. Injection of 5 mg progesterone on day 5, following 2 mg estradiol on day 4, significantly reduced the incidence of ewes with induced ovulations. Continued daily injections of 5 mg progesterone significantly reduced the incidence of ewes with maintained CL.

Estradiol appeared to initially depress progesterone production when measured by total luteal progesterone, which was significantly less in treated ewes on day 10, 15, or 22 than in control ewes on day 10. On day 37 (15 days after termination of estradiol injection) luteal progesterone levels were high compared to ovarian vein progesterone levels (table 4); this may indicate either progesterone retention by the CL or changes in blood flow. The results suggest that luteal progesterone production is decreased during estradiol injection but increased after termination of treatment in corpora lutea maintained for at least 2 additional weeks. This indicated that—at least in the ewe and under the conditions of this experiment—estradiol depresses CL function during treatment but results in a luteotropic influence following treatment termination.

Estradiol caused a decrease in pituitary LH on all days when compared to control ewes on day 15, but not on day 10. Jugular vein plasma levels of LH were increased when measured on day 10 (the only day measured) following estradiol injection (table 5). These results suggest that estradiol causes CL maintenance by releasing pituitary LH (Piper and W. C. Foote, 1968*; 1970*).

Some effects of different gonadal and gonadotropic hormones on luteal function were studied in mature cycling ewes, beginning at the first estrus following synchronization with 10 mg progesterone daily for 14 days. Table 6 shows the experimental design for this, and tables 7 and 8 give the results. On day 16 (first day of estrus = 0) CL weight was significantly decreased in all hormone treated groups when compared to the controls. CL were maintained to day 26 in ewes receiving estradiol–17β, estradiol valerate, and testosterone. CL weights at 26 days were heavier for the estradiol-17β and testosterone groups than for the estradiol valerate group.

The per cent of ewes with CL maintained morphologically to day 16 was, when judged grossly, significantly higher in ewes receiving estrogen, gonadotropin or testosterone than in control or progestin-treated ewes. Treatment with estrogen and with gonadotropin resulted in formation of new corpora lutea. Progesterone production, as measured by total gland content, decreased during treatment with estradiol-17β, estradiol valerate, HCG, and testosterone, but during treatment with progesterone or norethandrolone progesterone concentration did not decrease (W. C. Foote and Dermody, 1969* and unpublished data). These data agree with those of Piper and Foote (1969) for estradiol-17β. On day 26 of the study, total progesterone content in ewes from treated groups analyzed (estadiol-17β, estradiol valerate and testosterone) was similar to control CL on day 16. Progesterone concentration on day 26 was significantly higher in the treated groups than in the control group. The results show that at least these three steroids are effective in extending the functional life of the CL (Dermody and W. C. Foote, 1969*).

Daily levels of 50, 75, 100 or 150 μg of estradiol during the estrous cycle did not affect length of the cycle or the time from onset of estrus to ovulation. Daily levels of 200 and 300 μg decreased the length of the cycle, delayed ovulation, and increased secretion of mucus. Abnormally large follicles were found in some cows receiving 350 μg of estradiol per day, and 3.5 mg per day inhibited follicle growth and decreased the size of the ovaries. Levels of 150 μg per day prevented pregnancy when injected from the third to the sixteenth day following breeding, and 350 μg per day caused abortion when injections were initiated

on the 66th day following conception. Similar amounts administered after 100 days following conception had no effect (Rahlmann and Cupps, 1962*a**). The abnormally large follicles found following treatment of 250 to 350 μg daily were atretic, with degeneration of the glanulosa and partial luteinization of the theca interna (Rahlmann and Cupps, 1962*b**). High doses inhibited follicular growth and caused atresia in the small tertiary follicles.

Injection of up to 300 μg estradiol–17β or 12.5 mg progesterone directly into the ovary did not result in detectable alteration of ovarian structures observed at laparotomy or in estrous cycle length. In addition, the local steroid treatment did not appear to alter ovarian response to endogenous gonadotropin. These data were interpreted as supporting the hypothesis that systematically injected steroids act on the pituitary either directly or via the hypothalamus rather than directly on the ovary.

Effects of anti-LH

In another study, an attempt was made to measure the influence of LH directly on luteal function by injecting cycling, hysterectomized, and early pregnancy ewes with anti-ovine LH serum to inactivate circulating endogenous LH. Table 9 shows the design of the experiment, and tables 10, 11, and 12 show the results. Effectiveness of the anti-sera in inactivating and circulating LH is shown by the fact that the cycling ewes failed to return to estrus for longer than 55 days (time of slaughter) following treatment. Numerical but not always significant decreases in CL weight in the cycling ewes occurred at all three days measured and when data for the three days were combined the difference was significant. Treatment with anti-sera significantly decreased CL weight in hysterectomized ewes but not in ewes during early pregnancy. It appears from the data that LH is an essential luteotropic factor in cycling and hysterectomized ewes, but that it is not essential during early pregnancy—a period when CL is still required for its maintenance.

Changes in total progesterone and progesterone concentration in corpora lutea following treatment with anti-ovine LH serum followed a similar pattern to that of CL weight. This adds credence to the hypothesis that LH apparently plays an essential role in luteal maintenance in cycling and hysterectomized ewes, but is not essential during early pregnancy. This justifies speculation concerning the role of the uterus in producing luteolytic or anti-luteotropic factors which influence regulation of CL function (Dermody and W. C. Foote, 1969*b** and unpublished data).

Ovarian abnormalities

Two types of bovine ovarian abnormalities have been found after rectal examination. The first abnormality was noted in cows that exhibited no estrous cycles (Johnson, 1956*). Rectal examination of 23 anestrous cows indicated that the condition was due to a retained corpus luteum. These animals were given 50 to 75 mg diethylstilbestrol intramuscularly. All cows were in estrus within 24 hours after treatment and had subsequent normal regular cycles. Nineteen of the 23 cows conceived after three or more breedings; 33 services were required for a breeding efficiency of 69 per cent. There were no cases of prolonged estrus, abnormally frequent estrus, or other complications from treatment.

The other abnormality was a large luteal ovarian cyst (Johnson, 1960*; Johnson, 1966*b**) in cows 30 to 160 days postpartum. Of the 510 females routinely examined to determine their readiness to breed, 47 Holsteins and 27 Jerseys had either or both ovaries which contained a single, large (4 to 10 cm), fluid-filled cyst. Only five of the cows were in estrus when this condition was found, the other 69 cases were in the metestrus or diestrus. No symptoms of nymphonmania occurred. The cysts were very soft and could be manually expressed at an early stage; after about 15 days the cysts became hard and firm and could not be expressed. In two cases, cysts were removed for study and histological sections indicated they were luteal. A similarity in all cysts (noted by palpation) indicated that all were of this type.

In 14 of the 74 cases, treatments were not given and the cysts were not expressed; the cysts became hard and firm and could not be expressed. These 14 animals averaged 92 days to conception from the time the cysts were found. An average of three services (range: 2 to 6) was required for conception. Ten cases were treated with 60 mg of a luteinizing hormore while the cysts were stll soft; the ovaries returned to normal size in an average of 35 days (range: 17 to 46 days). The 10 cows conceived in an average of 70 days from the beginning of the treatment (range: 60 to 85 days). Two and two tenths services (range: 1 to 4) were required for conception. The large cysts were manually expressed in the remaining 50 cases as soon as they were found. All 50 cows conceived in from 10 to 68 days, with an average of 39 days after expression and no other treatment. One and one-half services (range: 1 to 3) were required for conception.

Thus it appears that this abnormality can be satisfactorily corrected by expression of the cysts, if found while still soft. When cysts become firm, treatment with luteinizing hormones may be satisfactory.

In Holstein heifers, exogenous progesterone, (12.5 or 25 mg per day) inhibited ovulation but allowed continued growth of one of the follicles in most of the animals. If ovulation occurred during the injections, formation of the corpus luteum was incomplete and luteal "cysts" were formed. In most cases these cysts appeared to have a normal function in that they formed progesterone from pregnenolone. If the follicles failed to rupture they changed and appeared to become cystic; when examined histologically, they resembled the follicles found in cows with spontaneous "cysts."

The blood of three cows with cystic ovaries was analyzed for steroids by chemical and chromatograyphic means. No testosterone, epitestoterone, or androstenedione could be detected, but a substance suspected to be 5a-androstane 3, 6, 17 trione was found. Corticosteroid levels at about 30 μg per 100 ml were found, half of which was cortisol. None of the corticoid was conjugated.

Cows with cystic ovarian degeneration show an increase in the width of the fascicular and reticular zones of the adrenal cortex, but the activity of the adrenal homogenates does not indicate a greater synthesis than found in normal open cows of similar breeding.

Three freemartin heifers were examined. Their blood contained corticosteroid-like material at 500 μg per 100 ml. None of this was cortisol, but some 19-hydroxycorticosterone was present. Incubation of the adrenals of these heifers with 21-C^{14}-progesterone yielded 20 to 30 per cent corticosterone, 4 to 12 per cent cortisone, 1 to 5 per cent deoxycorticosterone, and traces of a polar material resembling 19-hydroxycorticosterone.

Uterine studies

Endometrial biopsies from normal cows and cows that did not conceive were collected and analyzed histologically in hopes that a uterine change causing the abnormality might be detected readily. Biopsies were also taken from overiectomized animals treated with estrogen or progesterone, or both, (Cooper, 1961*; Sasser, 1963*; Johnson, 1965*a**), and microscopic measurements of endometrial nucous glands and epithelium were taken. These studies showed a cyclical nature of the endometrium, which was under the influence of ovarian hormones. Mucous gland size and the glandular epithelial height increased under the influence of estrogen. The gland lumen was small and the cells appeared to be storing mucin. Under the influence of progesterone the mucous gland size decreased and the epithelial height was lower. The glands appeared to be in a secretory state. Very high levels of progesterone or estrogen caused the gland size to decrease sharply. The endometria of five difficult breeding cows were indistinguishable from normal breeding animals at the corresponding stage of the cycle. Four cows with large (4 to 5 cm) luteal cysts were biopsied. In all cases, as long as the cyst remained intact the endometrium was similar to the 5 to 7 day post-estrus endometrium of the normal cow (Johnson, 1963*a**; Johnson, 1965*c**).

These studies do not provide any evidence that the endometrium was not

ready for implantation. However, it was noted that ovarian hormones have a definite influence on endometrial activity. It is possible that improper hormonal balances alter the endometrial activity, and thus prevent implantation.

Detection of estrus

All cows in the Idaho University herd were observed for signs of estrus five times daily for seven years (1961–1967). An improved record-system and rectal examination of the ovaries was used to detect estrus, and an estrus scoring system from 1 to 4 was employed. A score of 1 was given when no external signs of estrus were visible but estrus was confirmed by rectal palpation, a score of 4 was given to animals who stood to be mounted. The percentage of estrus periods scored 3 or less decreased from 26 per cent in 1961 to 5 per cent in 1965. These results indicate that proper training and more careful observation by the herdsman will enable him to detect more cows in standing estrus. They also indicate that the standing estrus period may be short and easily missed.

At the end of the fifth year (1965) reproductive performance based on average days calving to first estrus, first service, and conception and efficiency within a 365 day calving interval was 39.2, 73.5, 97.8 and 95.7 for 35 Holsteins, and 57.2, 87.6, 110.1 and 77.1 for 28 Holsteins the year before the improved management program began (1960). Corresponding values for 28 Jerseys in 1965 and 35 in 1960 were 39.4, 72.5, 93.2 and 96.4; 47.8, 78.2, 99.6 and 85.3.

During part of 1966 and all of 1967, palpation was discontinued and less rigorous observations of animals occurred even though the cattle were still observed at the same time periods and the record system was essentially the same. The average reproductive performance during these 2 years for the above parameters was as follows, respectively: Holsteins 51.9, 76.7, 121.3 and 92.1; Jerseys 50.3, 83.2, 103.5, 96.8. Performance decreased during the last 2 years. The data indicate that fewer animals were detected in estrus at the first estrus after calving and at the time of first service. This resulted in delayed conception. Efficiency remained about the same, which could be expected since cows that did not conceive readily were culled (Johnson, 1963*b**; Johnson, 1965*b**; Johnson, 1966*a**; Sasser and Ross, 1968*).

These data emphasize the necessity of rigorous herd observation to detect cattle in estrus. The average dairyman can improve estrus detection as much as 20 per cent by doing so.

Other hormones

An experiment was conducted to study the effects of insulin on ovarian activity, and ovarian response to pregnant mare serum (PMS) in beef cattle. Results indicated PMS treatment produced significant increases in ovarian activity, but insulin effects were nonsignificant. However, there was a suggestion that insulin treatment may have reduced the individual variation in ovarian response to exogenous gonadotropin (Bellows *et al.*, 1964*).

Injection of up to 20,000 guinea pig units of releasin directly into the ovary or corpus liteum of the cow had no effect on ovarian activity or estrous cycle length.

Ovulation control

Seventy-seven beef heifers ranging in weight from 308 to 563 kg were used to determine FSH treatments that would give a controlled increase in potentially fertile ova following synchronization of estrus. Estrous cycles were synchronized by feeding 180 mg medroxyprogesterone acetate (MAP) for either 9 or 11 days (first day of MAP feeding equal day 1) and an injection of 5 mg estradiol valerate given on day 2. FSH treatment levels were total dosages of 3.12, 6.25, 12.5 or 25.0 mg. All heifers were laparotomized and bilaterally ovariectomized at 60 to 72 hours after breeding. Oviducts were removed and ova recovered for microscopic examination.

Feeding MAP for 11 days with estradiol valerate given on day 2, followed by FSH injected twice daily on days 8, 9, 10, 11 and 12, produced the most predictable ovarian response. Total FSH

dosages of 12.5 mg or higher gave excessive ovarian stimulation. Average number of ovulations and fertilization rates were 1.1 and 92.9 per cent, 2.1 and 93.8 per cent, 8.0 and 79.4 per cent and 14.6 and 83.7 per cent for total dosages of 3.12, 6.25, 12.5 and 25.0 mg FSH, respectively. Statistical analyses indicated a linear dose-response relationship between dosage increase and the ovarian characteristics studied. No evidence was noted indicating a relationship between the size of heifer and response to FSH treatment. Dose-response effects indicated synchronization of estrus and total FSH dosages of 3.12, 12.5 and 25.0 mg depressed numbers of sperm per ovum. However, a total dose of 6.25 mg FSH did not result in this reduction, and this dosage overcame the depression in numbers of sperm per ovum caused by synchronization (Bellows *et al.*, 1969*).

GESTATION

Progesterone

The bovine CL contains relatively large quantities of progesterone and measureable amounts of 20β throughout gestation (Stormshak and Erb, 1961*; Erb and Stormshak, 1961*; Johnson and Erb, 1962*; Gomes and Erb, 1965*; Erb *et al.*, 1968*a**). Rate of excretion of estrogens in urine increasely slowly during the first half of gestation (Nelson and Smith, 1963*; Erb *et al.*, 1968*d**; Erb, Randel and Callahan, 1970*) and most rapidly preceding parturition (Nibler and Turner, 1929; Nelson and Smith, 1963*; Mellin, Erb and Estergreen, 1966*, Erb *et al.*, 1968*d**; Erb, Randel and Callahan, 1970*, Hunter *et al.*, 1969; 1970*). After the first month of pregnancy luteal weight and progesterone content did not change significantly. The content and concentration of 20β in the CL increased steadily during the first 3 months of pregnancy, declined and remained low for 3 months, increased from 199 to 238 days and declined during the last month ($P < .005$) (Erb *et al.*, 1968*a**).

Progesterone in blood plasma draining the ovary declined from a maximum level of 4.5 ± 0.8 μg per ml 14 days after conception to 2.6 ± 0.3 μg per ml during the second and third months. An increase was noted during the fourth month, but levels were lowest during 197 to 237 days of pregnancy (< 1.8 μg per ml) as compared to all other stages. The subsequent rise (average of 258 days) coincided with lower levels of progesterone and 20β in the CL and no change in the level of progesterone in jugular plasma. Although two increases occurred in level of progesterone in ovarian venous plasma, one at 52 days and again during the last month of pregnancy, the average linear downtrend was significant ($P < .01$) (Erb *et al.*, 1963*).

Levels of progesterone in peripheral plasma of pregnant cows tend to increase throughout gestation (Erb *et al.*, 1968*a**). Because ovarian venous plasma progesterone declined on the average, an extra ovarian source of progesterone may be available or more circulating progesterone is bound to blood proteins thereby resulting in a reduced rate of metabolism (Erb, Randel and Callahan, 1970*). Also, blood flow through the ovaries may increase during gestation, thus diluting the concentration of progesterone in ovarian venous blood. Although the CL remains active throughout pregnancy, weight and levels of progesterone in the CL do not reflect the decline in ovarian venous plasma or the increasing levels of progesterone in the peripheral plasma (Erb *et al.*, 1968*a**). Plasma progesterone declined 50 per cent within 2 weeks after ovariectomy of pregnant cows as compared to preovariectomy levels, but the rate of excretion of estrogen in urine did not change for at least 28 days after ovariectomy (Erb *et al.*, 1968*c**). The data demonstrate that the ovarian contribution is substantial for progesterone and probably unimportant for estrogen. It is known that the estrogen content of cotyledons of the fetal placenta increases rapidly during late pregnancy (Veenhuizen, Erb and Gorski, 1960*). The adrenal cortex hypertrophies during preg-

nancy and the gland does contain (Stormshak and Erb, 1961*) and releases progesterone (Balfour, Comline and Short, 1957).

In a more recent study, plasma progesterone and urinary estogen was measured throughout pregnancy (Erb, Randel and Callahan, 1970*). Plasma progesterone increased steadily from conception to day 43 of pregnancy, and then declined by day 65 and remained low through day 185. An increase occurred by day 200 and values for 8 of the 10 cows were higher at this time as compared to day 185 or day 230. It is during this period that ovariectomy generally does not cause interruption of pregnancy (McDonald *et al.*, 1953*; Estergreen *et al.*, 1965*). These changes are in general agreement with the earlier study where plasma progesterone was lowest at 115 to 144 days ($\bar{X} = 124$) and levels of progesterone in ovarian venous plasma were declining from days 95 to 238 (Erb *et al.*, 1968*a**). Plasma progesterone was highest by the end of the eighth month of pregnancy and declined significantly from 34 days to 3 days before parturition, with a further and more rapid decline during the last 3 days before parturition (Hunter *et al.*, 1969*b**, 1970*).

Ovariectomy and progesterone replacement

Forty-one cows of three dairy breeds were ovariectomized by laparotomy at 48 to 268 days of pregnancy. Twelve cows ovariectomized at 48 to 117 days of pregnancy aborted an average of 4 days after removal of luteal tissue. Ovariectomy of eight cows at 139 to 174 days resulted in abortion an average of 23 days later. Twenty-one cows ovariectomized at 200 to 268 days of pregnancy terminated in 2 to 74 days ($\bar{X} = 33$) at an average gestation length of 262 days. Based on previous history of these cows, the expected gestation length was 278 days. Of the 41 cows, 36 retained fetal membranes and 26 fetuses or calves died prepartum. Calving difficulties due to partial cervical dilation and uterine inertia, and postpartum metritis were common (Estergreen *et al.*, 1967*).

It appears unlikely that the abortions reported herein are related to surgical stress. Pregnancy was maintained to normal term at 279 days in one cow sham operated at 200 days. In three cows ovariectomized at 101 to 123 days, pregnancy was maintained during a 30 day progestrone treatment period and terminated 4 to 18 days after the end of the treatment. This indicates that abortion was due to lack of progesterone following removal of the corpus luteum.

Work to date shows that luteal tissue or exogenous progestin is essential for the maintenance of bovine pregnancy for at least 180 to 200 days. Thereafter, extraovarian sources of progesterone may be adequate in some cows to maintain a viable fetus, but the levels produced by these sources appear to be generally inadequate to support normal gestation lengths and normal parturition, including expulsion of the fetal membranes. Some ovariectomized cows maintaining pregnancy more than 264 days consistently had plasma progesterone levels near 10 ng per ml for periods of several weeks before parturition, and others were 2 to 3 times higher; the former value is similar to levels during estrus and to days postestrus in the non-pregnant cow (Plotka *et al.*, 1967*). Variation in levels of plasma progesterone between cows following ovariectomy indicates that cows vary relative to extraovarian sources of progesterone. As all ovariectomized cows maintaining pregnancy more than 264 days had abnormal parturitions and retained fetal membranes, it can be postulated that progesterone from the CL is essential for normal termination of pregnancy. The luteal component required is probably progesterone, as early withdrawal when pregnancy is maintained with exogenous progesterone results in retention of fetal membranes, whereas withdrawal at the time of predicted parturition does not (Johnson and Erb, 1962*; McDonald, McNutt and Nichols, 1954).

In another experiment, bilateral ovariectomy was performed on 22 dairy animals at 55 to 143 days of pregnancy. Exogenous progesterone in the form of Delalutin (17-alpha-hydroxyprogesterone-17-n-caproate) and Repositol progesterone

(progesterone in propylene glycol) was administered at various dosage levels and time intervals in an attempt to maintain pregnancy. Pregnancy was maintained following ovariectomy after 88 days with daily injections of 125 mg of Delalutin when the treatment was started at least 8 days before surgery. Daily injections of 50 mg of Repositol progesterone beginning 0 to 12 days before ovariectomy maintained pregnancy after the 66th day. Smaller daily injections of either Repositol progesterone or Delalutin were more satisfactory in maintaining pregnancy than larger less frequent injections. Neither Repositol progesterone nor Delalutin maintained pregnancy when 500 mg were injected weekly. When Repositol progesterone was withdrawn more than 6 days before parturition (expected at 279 days), the placentae were retained in all cases and were very difficult to remove 48 to 60 hours later. When repositol progesterone or Delalutin was withdrawn at 274–278 days, normal parturition occurred in 2 to 6 days.

Total progesterone and its concentration in the corpus luteum was significantly increased ($P < .005$) by administering progestins one to 12 days before ovariectomy. Δ^4-Pregnene-20β-ol-3-one was unaffected, being slightly higher in the untreated cows. There was also a consistent increase in progesterone concentration in the ovaries minus the corpus luteum for the treated cows, as compared with untreated cows ($P < .10$) (Johnson and Erb, 1962*).

Estrogen

Excretion of estrogen by pregnant cows decreased from days 7 to 9, increased by day 19, and remained constant through day 35 preceding implantation. A marked increase occurred by day 42 which is the approximate time of implantation in the cow (Amoroso, 1952); the principal urinary metabolite at this time is estradiol-17α. Following a decline by day 65, estrogen excretion (principally estradiol-17α) increased three times by day 245. Hunter *et al.*, (1970*) used a multiple regression analysis to determine changes in estrogen excretion and levels of progesterone in peripheral plasma during the last 28 to 34 days of pregnancy of 89 cows independent of breed, age and weight of cow, weight and sex of calf, time from parturition to expulsion of fetal membranes, and the linear and quadratic effect of variable length of gestation periods. Predominant changes during the last 34 days of pregnancy were increasing excretion of estradiol-17α and decreasing levels of progesterone. Though excretion of estone increased significantly on the average, the major change in rate occurred prior to 14 days before calving. One month before calving approximatley 50 per cent of the total estrogen excreated was estradiol-17α and 44 per cent was estrone. The proportion of estradiol-17α increased to 76 per cent at parturition and to 93 per cent at 0.5 days after calving.

Estrogen excretion in urine during estrus (day 0) and at 7 and 14 days after breeding has been studied at the Montana Station over a period of several years. Acid hydrolysis and fluorometric assay of the extracts were used to estimate total urinary estrogen in one experiment (28 cows—12 pregnant) and estrone and estradiols in a second experiment (15 heifers—7 pregnant). More recently the Indiana Station completed a study involving 35 cows (12 pregnant). Data from the Montana Station were transformed to express excretion as ng per mg urinary creatinine.

Table 13 shows that excretion of estrogen in urine was lower in all three experiments at 0, 7 and 14 days after conception as compared to similar periods after breeding and return to estrus (nonpregnant). Variation as indicated by the coefficients of variability between pregnant cows was considerably lower than for the nonpregnant cows in the three experiments. Variation among cows in the Indiana experiment was greater because of correction for method losses and the use of enzyme-acid hydrolysis, instead of acid hydrolysis alone, to maximize the yield of hormones extracted, especially estradiol-17α.

Cows and heifers were not significantly different in the Montana experiments but in each group the excretion by pregnant animals was lower as compared to the

nonpregnant animals ($P < .01$); also, in each experiment the animal within-group variance was significant ($P < .01$). Similar comparisons for the Indiana experiment were nonsignificant. For the combined data (table 13) average estrogen excretion was approximately three-fold greater at 7 and 14 days after breeding for the nonpregnant group as compared to the pregnant group. In an earlier study at Montana ($n = 25$), total urinary estrogens found in nonpregnant cows were 1.4, 1.8 and 1.3 times higher than found in pregnant cows at 0, 10 and 19 days after breeding, respectively (Smith and Vahedra, 1962*).

Table 14 shows that estradiol-17α was the predominant urinary metabolite in the pregnant group at estrus at 7 days, but estrone was highest at 14 days after breeding. These shifting patterns of excretion for the nonpregnant group suggest that the pathway of metabolism is different for cows returning to estrus following breeding. Randel *et al.*, (1968*) compared four pregnant and eight nonpregnant cows at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 14 days after breeding. Each of the eight cows failing to conceive had one or more daily excretion rates exceeding the highest level found among the cows which conceived. The total data on urinary estrogen excretion implicates these hormones as one cause of failure of conception. However, the mechanism which triggers abnormally high estrogen excretion is unclear. These results suggest that more detailed research should be directed towards the changes in steroid environment during the first 2 weeks after breeding.

Treatment with exogenous progestins

All cows of breeding age from the University of Idaho dairy herd were bred with semen from bulls of known fertility. After breeding, alternate cows were treated with 500 mg of progestin hormones while the other animals were not treated and were used as controls (Johnson *et al.*, 1958*). One treated group of 53 animals received 100 mg of a repositol progesterone intramuscularly on the 2, 3, 4, 6, and 9th day after the first breeding. This dosage and treatment period was determined from previous work by Johnson (1955*) in which 20 animals with four to nine unsuccessful breedings had a breeding efficiency of 80 per cent. Sixteen of these females conceived on first breeding, there on second, and one on third breeding after such treatment. Thirty-six of the 53 animals conceived to first service for a breeding efficiency of 67.9 per cent. Thirteen conceived to a second, two to a third, and two to a fourth service without further treatment. The 53 animals conceived on a total of 76 services, for a breeding efficiency of 69.7 per cent.

Another treated group of 17 animals received 500 mg of 17-alpha-hydroxyprogesterone-n-caproate (Delalutin) intramuscularly on the second day after breeding. Thirteen of the animals conceived to first service, for a breeding efficiency of 76.5 per cent. The other four animals returned to estrus on the 21st day, were rebred, and conceived to that service.

Sixty-nine animals were not treated and were used as controls. Twenty-nine coinceived to first service for a breeding efficiency of 42.0 per cent. Twenty-six conceived to a second, nine to a third, and five to a fourth service, for a total breeding efficiency of 53.9 per cent.

The combined treated group had a breeding efficiency of 72.7 per cent (70 conceptions from 97 services). The control group had a breeding efficiency of 53.9 per cent (69 conceptions from 128 services). Comparing the breeding efficiencies from the first two services from each group, the results are 72.5 per cent for the treated group and 50.5 per cent for the control group. The difference in breeding efficiency between the treated and control groups was significant at the 0.05 level by chi-square.

These data show that exogenous progestin hormones will improve reproductive efficiency when administered within 9 days after breeding.

Effects of high environmental temperatures

Research into the seasonal breeding problems of dairy cattle in Arizona (Stott, 1961) consisted of studying breeding records (46,236 receipts) obtained from

an artificial insemination organization. The information was confined to cows bred in the Salt River Valley over a 10-year period. Semen used during this time was collected in Phoenix, Palo Alto, and in Columbus (Ohio). Data for the study were transferred from the breeding records to I.B.M. cards and sorted according to breed, bull, source of semen, date of breeding, and number of services for conception.

Two main seasonal depressions in fertility were found to occur during the year, one during the summer months (21 per cent), which included June, July, August and September, and the second during winter months (9 per cent), varying from year to year but generally occurring during December, January and February. Two factors point to the cow as the major contributor to summer seasonal depression in breeding success: (1) the depression in fertility was comparable for all sources of semen (Columbus, Ohio; Palo Alto, California; and Phoenix, Arizona) when used on Arizona cows during the summer and (2) cows bred outside Arizona during the same period and with semen from the same bulls showed a high rate of fertility during the summer months, contrasting with the lowered breeding efficiency within the state.

Differences in seasonal fertility according to breed were also found. Data from the Jerseys showed no depression during the summer months, but Guernseys and Holsteins had deep declines in fertility.

An investigation of time of ovulation and anovulation has been conducted (Williams, 1962*). No particular relationship could be found between these two factors and lower seasonal breeding efficiency. To investigate other conditions that could be involved with the cow breeding efficiency in hot weather, a herd of 406 two-year-old Holstein cows which calved from March through October was used (Stott and Williams, 1962*). The animals were rebred at the first estrus after 60 days post-partum. Careful pre-breeding examinations indicated the cows had no greater incidence of infection or anatomical abnormalities of the reproductive tract than would be expected during any season of the year. They were all bred with stored frozen semen to avoid confounding seasonal differences attributable to semen.

Pregnancy examinations were made by rectal palpation at 35 to 41 days following insemination if the animal did not return for service. A second examination was made at 72 days. Unless the animal showed heat or indications of abortion, no further examinations were made. The examinations were planned to help determine the rate of fetal mortality after 35 to 41 days gestation, and to give some indication of embryonic loss before this time relative to ambient temperature and humidity.

Breeding efficiency declined during the summer months. Each month from June through September, the number of cows returning for service by 35 days after insemination increased. Also, there was an increase in the number of non-return animals diagnosed nonpregnant at the first pregnancy examination (35 to 41 days post-insemination). This depression in breeding efficiency was most severe in August when, of 111 animals bred, only 19 (17.1 per cent) carried viable embryos. Although 65 head (59 per cent) did not return for service, only 29 per cent of these were diagnosed pregnant. Cows bred in September had the lowest non-return rate (53 per cent) by 35 days. After September both non-returns and the percentage of cows pregnant at 35 to 41 days improved, reaching levels that could be expected under favorable climatic conditions. The data would indicate that a low rate of fertilization and a high rate of embryonic mortality are the major factors associated with low breeding efficiency.

A large percentage of the cows bred during the summer months did not return to estrus by 35 days; and were nonpregnant at the 35 to 41 days. These animals averaged 48 days to the next estrus.

Thyroid: parathyroid studies

In the bovine, low reproductive efficiency observed during periods of high environmental temperature could be due to depressed thyroid secretion. Bovine thyroid activity is known to be affected by prevailing temperatures, and hypo-

thyroidism has been shown to affect fertility in females of many mammalian species.

Usually, removal of the thyroid results in cretinism or myxedema or both after prolonged periods. Transient-like hypothyroidism (which would be produced during summer) should be reproducible by removing the thyroids during other seasons. Therefore, it seemed expedient to remove the thyroid from cows and breed them soon after to determine whether a lack of thyroid secretion alone could be a factor in lowering seasonal breeding efficiency (Williams and Stott, 1966*). As effects of hypoparathyroidism on reproduction on the bovine are unknown, some thyroidectomized animals also were parathyroidectomized.

Of 15 first services over two breeding periods, 80 per cent resulted in conception. Two of the parathyroidectomized animals were not able to carry their fetuses to term in either of the two breeding periods. Severe hypocalcemia appeared to cause abortion in each case. Lack of mammary development and subsequent low daily milk production (6 to 12 pounds), occurred in the parathyroidectomized heifers, whereas the two thyroidectomized heifers reached daily production of 45 to 32 pounds. Milk production in the mature parathyroidectomized cow was not altered.

Results of this study do not support the theory that hypothyroidism is the cause of low breeding efficiency in the dairy cow during hot weather. In fact, the 80 per cent conception on first service was excellent and contrasts sharply with previous results obtained under the adverse summer climatic conditions found in southern Arizona.

Both exogenous progesterone and pregnant mare serum (PMS) were used to determine if breeding efficiency might be improved during the summer (Stott and Williams, 1962*). First service lactating dairy cows were injected (I.M.) with 500 mg of progesterone (Repositol progesterone) at the time of insemination, 12 hours after start of standing estrus. Every other first service cow inseminated was used as a control. The same procedure was used on a second group of cows, except that treatment was 10 days after insemination. Animals not returning to heat by 27 days were assumed to have conceived and were examined for pregnancy at 35 days.

When injections of 500 mg of progesterone were given at the time of insemination as on day 10 the estimated rate of embryonic death was 49 per cent in both groups compared to 31 per cent and 46 per cent, respectively; for the control groups (nonsignificant) progesterone was ineffective in preventing low breeding efficiency during hot weather when injected only once (500 mg) on day of insemination or 10 days later.

In another experiment, attempts were made to speed up ovulation time to determine if this would improve breeding efficiency. As cows came into heat, one-third were injected with progesterone (10 mg), one-third with PMS (25 i.u.), and one-third were used for controls. The ovaries of each animal were examined at the first signs of estrus and at 12-hour intervals thereafter until 48 hours had elasped. The examinations revealed the position of the mature follicle and the approximate time of ovulation. Pregnancy examinations 35 days after breeding showed no significant differences between groups.

Controlled environment studies

A controlled environmental chamber large enough to hold four cows was constructed to simulate as closely as possible Arizona's average temperature and humidity during the hot summer months. In some of the initial work, lactating dairy cows were placed in the chamber at different periods from the beginning of estrus to 23 days post-insemination. Temperature in the chamber was raised to 105° F. by 9:00 A.M. each morning and maintained until 5:00 P.M. when it was lowered to 80° F. and held until morning. The relative humidity was also controlled at 42 to 44 per cent during the day and at 46 to 50 per cent during the night corresponding to the lower temperature. Light was on for 12 hours and off for 12 hours.

Lactating cows were unable to sustain pregnancy when exposed to the high temperature prior to 16 days after being

bred. Judging by late returns to estrus and observations during rectal palpation and at slaughter, a high rate of embryonic death seemed to occur. Non-lactating cows and heifers, however, seemed to conceive and maintain pregnancy after exposure to high ambient temperature.

As part of another experiment (Borges, 1964*) that required sacrificing the animals at 35 days after breeding, a comparison of reproductive performance was made between lactating and non-lactating cows whose body temperatures were raised to equivalent levels in the temperature-controlled chamber. The animals were placed in the chamber at the time of estrus, bred, and held for 24 hours. Ambient temperatures in the chamber were 105° F. to 107° F. with relative humidity at 50 per cent. Lactating cows reached maximum increases in body temperature (2° to 5° F.) much sooner than did non-lactating cows and heifers. It was necessary to raise the ambient temperature slightly for the non-lactating animals in order to increase their body temperature to be equivalent to that of lactating cows.

Eleven lactating cows and nine non-lactating cows were exposed to the high temperature. In addition, 12 non-lactating cows not heat-treated were bred at the same time for controls. All 11 lactating cows were found to be without embryos at 32 days after insemination, as compared to only two out of nine non-lactating cows. Fertility in the non-lactating cows exposed to high temperature is very similar to fertility in the controls, where eight out of 12 had normal embryos. Two control cows had embryos that were necrotic at time of slaughter. These results again indicate that lactation has some influence on fertility related to thermal stress.

With exidence from the previous experiments that lactation in some way predisposes poor reproductive performance in dairy cows subjected to high ambient temperatures, an attempt was made to determine whether this was true under natural herd conditions during the summer. One hundred and eleven Holstein heifers were bred during the interval from July through September, the most adverse period for reproduction. Forty-six head settled on the first service. During the same period 150 lactating cows were bred in the same herd, with only 14 head being confirmed pregnant. The calculated 41 per cent successful breedings in the heifers is obviously different from that of the cows (9 per cent), though somewhat different than would be expected under normal conditions. It is evident, however, that lactation is involved in the thermally induced reproductive problem, thus suggesting endocrine involvement.

Any abnormal secretion of gonadal hormones would probably be reflected in the appearance of uterine tissue. With circumstantial evidence pointing to an endocrine involvement, a study was made (Borges, 1964*) of uterine tissue taken from cows sacrificed at 14 to 32 days post-breeding following thermal stress. All uteri recovered at 14 days post-breeding, whether the cow had been heat-treated or was acting as a control, had uniform development of both uterine horns. In the animals that had either no embryos or degenerating embryonic tissue (which included all eleven of the lactating cows heat-stressed and two of the non-lactating heifers), the histology of the uteri was variable. In some cases the tissue was similar in many respects to the 32-day normal gestating cow, having highly convoluted uterine glands and eroded flattened epithelial cells lining the endometrial surface.

The variability in the histology of the uterine tissue from the experimental cows carrying abnormal embryos at 32 days post-breeding, does not allow for drawing conclusions as to what might be affecting the embryonic loss. It is possible that variation in appearance of tissue reflects the stage of regeneration following loss of the embryo or a distinct stage of a new estrous cycle.

Efforts have been made to identify the stage of development or fertilization when reproduction is adversely affected by high ambient temperature. Earlier experiments indicated a critical period occurred during the first 10 days after insemination.

Other studies reported in the literature for different species indicate the first four days after breeding as the critical period.

Effects of cooling

A barn which could be cooled was constructed (Wiersma and Stott, 1966*). Cows were placed in the cool environment from the beginning of estrus up to 188 hours after breeding during the summer. During the first summer 26 per cent of 92 cooled cows conceived, as compared to 17 per cent of the controls. Confinement periods of 4 to 6 days the following summer in the cooled barn again showed no improvement in breeding efficiency as compared to controls. The implication is that cows not cooled prior to estrus have low breeding efficiency even when cooled for the first 6 days after estrus. In contrast, unstressed controls show normal breeding efficiency unless stressed after beginning of estrus. This shows that thermal stress can cause decreased breeding efficiency before as well as after breeding. It seems reasonable to suspect that the endocrine system may be involved. An attempt was made to change the microclimate by modifying existing shade to include evaporative cooling. The cooling system maintained the modified shade 8° to 10° F below that of control uncooled shade. Even so, temperatures would ofter approach 100° F under the modified shade during the summer.

Two groups of lactating Holsteins, 43 using cooled shade and 43 using uncooled shade, were started on experiment in June. Breeding efficiency of the cows using cooled shade was 58 per cent as compared to 35 per cent for conventional shade during June, July and August. A pregnancy percentage of 58 for cooled cows is an acceptable rate under moderate environmental conditions. The difference in breeding efficiency between the cooled and uncooled cows makes it clear that a modest reduction of ambient temperature during the peak of hot summer days can have a considerable effect on fertility.

Steroid hormone levels

The symptoms of animals showing infertility during hot weather or when exposed to thermal stress in the environmental chamber, suggested that progesterone might be involved. Secretion of either excessive or insufficient amounts could well result in incompatibility of the uterus to the embryo. An imbalanced progesterone secretion could also cause the prolonged estrous cycles observed in animals bred but not pregnant. Progesterone studies (Moody, 1964*) were initiated in cows exposed to high ambient temperatures to determine whether this might be the case. Forty-five dairy heifers and cows were bred and then assigned to one of four groups. Twenty-two were placed in the controlled temperature chamber for 24 hours, beginning on the day they were bred, and then heat-stressed for 24 hours. Eleven of these were sacrificed on the 14th day after breeding, and the other eleven on the 32nd day. The other 23 animals were bred and used as controls, half being sacrificed at 14 days and the balance at 32 days post-breeding. Animals in the 32-day control group that returned to estrus before the slaughter date were rebred until they went the 32 days without showing estrus.

When the animals were sacrificed, the corpus luteum and adrenal glands were weighed and samples taken for progestin analysis. Progestin levels in the ovaries of the animals subjected to thermal stress were different from levels in the ovaries of control animals taken at the same time of the estrous cycle or gestation. In cows and heifers heat-stressed and sacrificed at 14 days post-breeding, progestins were lower (43.8 μg per gm) than control animals (51.1 μg per gm), by a significant amount. Those sacrificed at 32 days post-breeding differed also from controls, depending upon whether they were able to sustain gestation after the thermal treatment. The pregnant heat-stressed animals had a progestin ovarian content (53.7 μg per gm) less than that of the control pregnant animals (62.9 μg per gm) but not significantly different, whereas non-pregnant heat-treated animals were much lower (37.1 μg per gm). This difference was highly significant. Ovaries from control animals not found carrying viable embryos at 32 days post-breeding had

exceedingly high progestin concentrations (105.4 μg per gm).

Analysis of the adrenal glands for progesterone revealed a difference also. When the animals had been thermally stressed, concentrations in μg per gram of adrenal tissue ranged from 1.8 to 5.0 for heat-treated groups, and from 0.4 to 1.9 for control groups.

It is not surprising that adrenal content of progesterone is higher following stress. Of importance is whether the adrenal progesterone is secreted into the blood stream where it could be carried to target organs to affect reproduction—the lower ovarian content of progestins in the thermally stressed animals would indicate that this is happening. Normally, progesterone is synthesized in the adrenal cortex and this is thought to be one of the cyclic steps in the biosynthesis of corticosteroids. More direct evidence of its secretion into the blood would have to come from measuring blood concentration in stressed animals.

To determine if progesterone is secreted into the blood from the adrenal glands following or during thermal stress, three cows were ovariectomized and exposed to high temperature (105° F.) in a climatic control chamber for 24 hours (Stott, 1969*). The animals were bled every 6 hours during the stay in the chamber then every 24 hours thereafter for 21 days. By the first 6 hours in the thermal chamber, plasma progesterone increased above pretreatment levels (10 to 20 ng per ml) and continued to rise reaching a peak of near double pretreatment levels (28 to 52 ng per ml) by 24 hours. The plasma concentration then declined rapidly to unmeasurable amounts. A second increase then began, with a sustained high level of plasma progesterone for 3 to 4 days; at the end of this period, the plasma progesterone concentration became very low (0 to 3 ng per ml) and remained so thereafter. With the thermal treatment of intact control cows, the same results occurred. One major difference, however, was that plasma progesterone was sustained at a higher level after the acute effects of the stress had dissipated, indicating that the ovaries were actively secreting progesterone.

Plasma cortisol concentration followed the same pattern as progesterone, increasing and decreasing in response to the thermal stress, though at much higher levels (45 to 65 ng per ml). Pretreatment patterns of plasma progesterone and cortisol variations during a normal estrous cycle indicated that both followed the same cyclic pattern—which suggests that hypophysial release of gonadotropins and adrenalcorticotropins are controlled by the same mechanism. If this is true, excessive corticoid secretion during stress obviously could inhibit pituitary release of gonadotropins to the extent that normal reproduction could not be sustained. Evidence of this are the low plasma progesterone and cortisol concentrations found in experimental animals following acute response to thermal stress.

It is often inferred that long seasonal stress on man or animals due to weather (particularly hot weather) results in excessive adrenal secretion in order for the organism to survive. But the reverse is true: plasma cortisol from dairy cattle (Stott, 1969*) taken during the hot summer months is extremely low (0 to 10 ng per ml), and cattle having the most protection from heat and sun have the highest level. This has been determined by a study of lactating cows being shaded and cooled by artificial means. Actually, plasma cortisol concentration has proved to be a good index of the comfort of dairy cattle during hot weather. When the weather moderates in the fall, plasma cortisol concentration increases in the average to nearly triple the summer level (20 to 30 ng per ml). Some 215 lactating cows were bled each month throughout summer to determine plasma cortisol concentrations. The fact that it is secreted at a low level, and that apparently there is a relationship between adrenal activity and pituitary secretion of gonadotropins, seems to point to the low output of gonadotropins from the pituitary as the possible cause of low breeding efficiency in hot weather.

The possible sequence of events following the low gonadotropic output would be ovarian suppression, with limited progesterone secretion resulting

in poor conception and high embryonic mortality. All of these reproductive anomalies occurred following thermal stress. Lower levels of plasma progesterone in summer, particularly in cows of low breeding efficiency, have also been demonstrated (Riggs, 1970*). Thirty-eight cows were tested in August and 32 were tested in May. Blood samples were taken on the day of breeding, at the time ovarian output would be expected to be at a low ebb. Cows that conceived had higher levels (.78 ng per ml) than those that did not (.76 ng per ml) in August, though the difference was not significant. Samples taken from cows in May were much higher (1.24 ng per ml) with the cows conceiving (1.39 ng per ml) having significantly higher concentrations than those that did not (.95 ng per ml).

Placental hormones

Placental tissue from cows at approximately 5 months of pregnancy was analyzed unextracted or following extraction with amonium sulphate or phosphate buffer solutions (Lunnen and W. C. Foote, 1967).

The various pituitary levels of LH in cattle measured by the OAAD method and RIA method are shown in table 15 (Svejda and W. C. Foote, unpublished report). These results are based on small numbers of animals of different ages and breeds, but provide some information on levels of pituitary LH during gestation and at 20 days postpartum. Pituitary LH reaches a peak at 2 to 4 months of gestation. The LH level may be very low near the end of gestation. The LH level of the pituitary at 20 days postpartum appears to be about equal to that during 1 to 2 months gestation. Pituitary levels do not necessarily reflect the circulating level. Jugular serum levels of LH for different stages of gestation are also shown in table 15. These results show low circulating levels of LH at all times measured during pregnancy with no discernible changes (Svejda and W. C. Foote).

Weeth and Goldman (1958*) were unsuccessful in finding gonadotropic activity in bovine placental tissue using changes in the testis weight of cockerels as a measure of activity. Although this method was as good as any available, it lacked sensitivity. Later work (W. C. Foote and Kaushik, 1963*) showed that bovine placental tissues depleted ovarian ascorbic acid, which suggested that they contained LH-like activity.

Tables 16 and 17 (Lunnen and Foote, 1967) indicate results of analysis for LH-like activity in phosphate buffer extracts of bovine placenta. These results are based on ventral prostate response and show that LH-like activity is present in all four placental regions studied (fetal cotyledonary and intercotyledonary, and maternal cotyledonary and intercotyledonary). The results also show that activity is present in all of the column fractions except fraction 4. Radioactive steroids (dihydroepiandrostone and progesterone) incubated with extracts and subsequently subjected to column separation came off the column in fraction 4. Therefore, any contaminating steroids did not appear to affect ventral prostate response. Ammonium sulfate fractions of fetal cotyledons were also denatured by heat prior to bioassay, with resulting loss of activity (Lunnen and W. C. Foote, 1967). LH-like activity was measured in certain fractions of maternal jugular and uterine blood and umbilical blood following phosphate buffer extraction (table 17) (Lunnen and W. C. Foote, 1967). The presence of LH-like activity has also been shown at different stages of gestation and after column separation by Sephadex G-100 and G-50 followed by G-75; the initial extractions were made with ammonium sulfate. Activity was shown by both OAAD and RIA methods. Results from RIA produced apparent non-paralleled cross-reactions to NIH-LH-B, indicating the possible existence of a non-LH, LH-like gonadotropin (Niswender, Foote and Svejda). Any interpretation of activity measured by RIA must wait for further analysis. Analysis of sheep placental extracts with OAAD suggest LH-like activity is also present in this species (Svejda and W. C. Foote).

The question of whether the LH-like activity measured in the placenta is of placental origin or is stored pituitary LH remains to be answered. Research, on placental gonadotropin has failed to es-

tablish any specific source, although it appears to have higher concentrations in the fetal intercotyledonary tissue. Also, activity has been shown in column fractions with estimated molecular weights of from 180,000 to less than 5,000. There are no published data showing that the uterus stores or concentrates LH. Such a hypothesis might help explain some mechanisms of control phenomena such as CL function. A search of the literature reveals no data showing that gonadotropins act directly on the uterus to elicit a response as is the case with other tissue such as the ovary. Cordozo 1967) found no uterine response to PMS in the ewe when measured in terms of certain aspects of histology and biochemistry.

PARTURITION

Estrogen-progesterone ratios

Cows having gestation periods of 280 to 286 days excreted more total estrogen at parturition than cows having shorter or longer periods of gestation. These differences occurred during the last 10 to 14 days before calving (Hunter *et al.*, 1969*, 1970*). Omitting cows bearing twins, it was found that those calving in less than 280 days after breeding were characterized by a rapid decline in plasma progesterone during the last few days before calving. Those calving in 280 to 284 days did not decline significantly in plasma progesterone, but there was an exponential increase in excretion of estradiol-17α. In gestations longer than 284 days, progesterone decreased gradually during the last 34 days of pregnancy and rate of excretion of estrogen increased gradually. By parturition the ratios of progesterone to estrogen (ng progesterone divided by μg estrogen per mg urinary creatinine) were similar for each group. Days 7, 10 and 14 precalving represented approximately the same average time after conception for the three groups and the decrease in ratio of progesterone to total estrogen from these periods to parturition was 2.3 to 2.7 times, respectively. Progesterone:estradiol-17α represented the major cause of shift in ratios in all three groups (Hunter *et al.*, 1970*).

These data suggest that the rate at which estrogen reaches overdominance differs between cows, and that it is related to variable rates of change in level of plasma progesterone and excretion of estrogens in urine. The differences in level of progesterone associated with length of gestation and breed explain what appeared to be a disagreement between two earlier reports. Short (1958) reported a decrease during late pregnancy but Erb *et al.* (1968*c**) did not observe a significant decline. Judging by the data of Hunter *et al.* (1970*) it is questionable that progesterone always decreases during the last few days preceding parturition, as only those cows calving in less than 280 days after breeding showed a significant decrease during the last 3 days before calving.

Calf losses

A study was conducted on 3049 parturitions to determine causes of calf losses at birth and during the first 30-day postnatal period. A total of 143 calves were subjected to detailed postmortem examinations. Three-year-old primiparous heifers had a significantly higher loss than did 4 or 5 to 10 year-old dams. Seventy-one (57.3 per cent) of the calves lost at birth died because of delayed and difficult parturition. More males than females required assistance at birth, were lost and exhibited postmortem findings indicative of injury due to prolonged parturition. Data indicate that improved management procedures could reduce calf losses (Anderson and Bellows, 1967*).

A study involving 95 Hereford and 103 Angus primiparous dams has been conducted on some factors associated with calving difficulty. All dams were bred via artificial insemination to produce reciprocal crossbred calves using one Angus or one Hereford sire. Factors studied included size of pelvic opening of the dam, and sex and birth weight of the calf. Precalving body weights and measurements of height and width of the pelvic opening

were studied 5 days prior to the beginning of the 75 day calving season. Calving difficulty was scored numerically ranging from 1 (none) to 4 (extreme). Abnormal presentations (4 calves) were not included in the analyses. Calf sex was coded 1 for males and 2 for females. Average precalving body weights and pelvic areas were 378 kilograms and 247 square centimeters and 359 kilograms and 251 square centimeters for Hereford and Angus dams, respectively. Average birth weights of male and female calves were 33.3 and 30.7 kilograms and 31.2 and 30.2 kilograms from Hereford and Angus dams, respectively. Calving difficulty was more frequent with male offspring than with female (65.1 vs. 30.7 per cent, $P<.01$) regardless of breed of dam.

Correlations between calving difficulty and pelvic area of the dam and sex and birth weight of the calf were –.18 ($P<.10$), –.47 ($P<.01$) and 0.54 ($P<.01$) for Hereford dams and –.22 ($P<.05$, –.26 ($P<.01$ and 0.48 ($P<.01$) for Angus dams. Standard partial regression coefficients of calving difficulty on pelvic area of the dam, sex and birth weight of the calf were –.24 ($P<.05$), –.26 ($P<.01$) and 0.48 ($P<.01$) for Hereford dams and –.36 ($P<.01$), –.13 ($P<.10$) and 0.56 ($P<.01$) for Angus dams (Bellows *et al.*, 1969*).

The relationships among the area of the pelvic opening, hip width, rump length and body weight were studied prior to calving in 3-year-old primigravid Hereford Heifers. Statistical analyses indicated highly significant associations and cause-and-effect relationships of the three external measurements on pelvic area. Associations found suggest that larger skeletal size is indicative of larger pelvic openings (Bellows *et al.*, 1965* and Bellows, 1968*).

POSTPARTUM

Effects of steroid hormones

The influence of progesterone or estradiol treatment, or both, given days 5 to 17, 12 to 25 or 18 to 35 after calving, on the lengths and variations of the postpartum intervals studied have been reported (Foote and Hunter, 1964*). This study was partially repeated and the results confirmed in a different year (Saiduddin, Quevedo, and Foote, 1968*) by similarly injecting cows on postpartum days 5 to 15, 14 to 24, or 23 to 33 with progesterone and then giving each cow a single estradiol injection 2 days after the last progesterone injection (table 18).

Cows given estradiol alone or after progesterone treatment resumed ovarian activity earlier after calving than did untreated or progesterone treated cows. The treatment given earliest after calving caused the earliest ovulations. However, cows treated at "mid" postpartum anestrus had the shortest intervals to conception because of a higher conception rate at first preceded by progesterone in hastening the onset of estrus and ovulation; conception occurred earliest in cows given both hormones.

Both progesterone and estradiol injections tended to decrease the variations of the intervals to estrus, ovulation, and conception. Most of the treatments did not significantly affect interval to uterine involution, although some treatments tended to decrease this interval. Neither the length nor variation of the first postpartum estrous cycle of cows failing to conceive at first estrus appeared to be affected by any of the treatments given.

In an additional investigation to determine the influence of estradiol alone (Foote, 1969*), a single injection of 10 mg estradiol-17β given 9 to 15 days (average = 13 days) after calving, shortened the postpartum interval to first ovulation from 49 days for the controls to 33 days. The corresponding figures for interval to estrus were 60 days in the controls and 34 days in the treated animals ($P<.05$). Postpartum intervals to uterine involution and to conception were not significantly decreased by treatment. Although this early estradiol treatment tended to hasten ovarian activity after calving, only a few treated cows responded by ovulating within the first week after treatment. Other cows received this estradiol treatment on postpartum day 12 and again on day 17 to test for a priming effect of the first treat-

ment. However, no additional response was observed. A test for estradiol dosage effect showed no difference between 10 and 20 mg when given 25 days postpartum.

The results of treating Holstein cows in three different herds with 10 mg estradiol-17β 12 days after calving (Foote, 1969*) indicates that these animals are more responsive to this steroid during early postpartum anestrus than are beef cows. Table 19 summarizes these data. None of these cows was bred during the experiment. This estradiol treatment usually stimulated early ovulation, often within 1 to 2 days, although there was considerable variation in response between herds and between cows within herds. In some cases the animals showed no response to treatment. Normal estrual cycles tended to follow induced ovulations. Any differences between beef and dairy cows in response to estradiol may be due to genetic differences, nutritional differences, or the frequent suckling stimulus in beef cows versus twice a day milking by machine in dairy cows. The level of milk production was not considered in this experiment.

Gonadotropin levels

Pituitary glands and ovaries were recovered following slaughter at 5, 17 and 30 days after calving. Pituitary FSH was measured by the HCG augumentation method (Steelman and Pohley, 1953) at UCLA, and LH was measured by the OAAD method (Parlow, 1961) at UCLA and Nevada. The ovaries were observed for degree of follicular development. The results suggest that pituitary LH activity is nearly depleted at calving time but increases as the postpartum interval progresses. FSH concentration was relatively high when measured 5 days after calving and decreased during postpartum anestrus, thus showing an inverse trend to that of LH. A concurrent increase in ovarian follicular development suggests that FSH may be released during postpartum anestrus to stimulate follicular growth preparatory for resumption of estrual cycles.

Beef cows were killed the 275th day of gestation (approximately 1 week to 10 days before parturition), the day of calving, 20 days after calving and days 0 (the day of estrus), 1, 16 and 19 of the first postpartum estrous cycle. Pituitary LH and FSH levels were assayed by the OAAD and HCG augmentation methods at UCLA. Ovaries were observed for follicle and corpus luteum development; corpora lutea were analyzed for progesterone activity at the Washington Station.

The trends were similar to those shown in table 18 for comparable slaughter times (table 20). Corpora lutea of pregnancy were completely regressed by day 20 so that no detectable levels of progesterone were present in the ovary until after the first ovulation. The trends of pituitary LH and luteal progesterone during the first estrual cycle were generally inverse to each other. Values for other stages of the estrual cycle have been reported (Quevedo, 1965*).

The relationship of pituitary gonadotropin levels and changes to ovarian and uterine functions were studied in two groups of cows. Cows were ovariectomized or hysterectomized, or both, the day of calving, and their pituitary glands (and ovaries and uteri when applicable) were obtained 20 or 70 days later. The pituitary glands were assayed for FSH by the HCG augmentation, and for LH by the OAAD assay at the Utah Station; LH was also measured by the radioimmunoassay at Michigan for a comparison between assay methods.

The results of the first year's study showed that ovariectomy the day of calving caused a decrease in pituitary LH and an increase in FSH 20 days later, as compared to unoperated controls. By day 70, LH levels of ovariectomized animals had increased to near the control levels and FSH was unchanged from day 20. Hysterectomy appeared to have no effect on pituitary gonadotropin content. Ovariectomy did not substantially modify uterine morphology.

Table 21 shows the results of the second year's study, in which only LH was measured and all cows were killed 20 days after calving. The data for the 2 years are similar. Good agreement was found between the OAAD assay and the radioimmunoassay for LH.

Records from both beef and dairy cows were used to test for uteroovarian relationships during the postpartum interval (Foote and Peterson, 1968*). It was found that ovarian activity (follicular development and ovulation) first occurred in the ovary opposite the side of the recent pregnancy in a significant majority of the observations. This tendency was greater in the dairy cows. The proportion of cows with ovarian activity on the side opposite that of recent pregnancy tended to decrease as the length of the interval from calving to ovarian activity increased. However, the average length of the interval from calving to follicular development or ovulation did not differ significantly between "same side" and "opposite side" animals. Cows served at the first ovulation after calving had better conception rates when this ovulation occurred on the side opposite the previous pregnancy. This difference between the two sides was greater for cows whose uteri had not yet involuted at the time of service.

Steroid hormone levels

Corpora lutea were obtained from 8 dairy cows (Erb *et al.*, 1968*) and 9 beef cows (Nevada) 1 to 4 days postpartum. The drop in progesterone concentration immediately postpartum is dramatic. Total progestins at 1 to 4 days postpartum was less than 5 per cent of the level found in corpora lutea of pregnant cows shortly before parturition. The average CL weight declined only 20 and 40 per cent in beef and dairy cows sampled during this same interval, while concentration of progesterone and 20β declined 80 to 94 per cent. This appears to indicate that there is a rapid postpartum regression of luteal function with a much slower regression in CL size.

Cows which were ovariectomized 1 to 4 days postpartum ($\bar{X} = 2.3$ days) had mean progesterone levels of 12.41 ± 1.0 ng per ml in the jugular venous plasma, and 0.4 ± 0.2 μg per ml in ovarian venous plasma. This compares with 36.5 ± 3.5 ng per ml in jugular plasma and 3.0 ± 0.8 ng per ml in ovarian venous plasma of cows 247 to 284 days pregnant (Erb *et al.*, 1968*a**). The mean plasma progesterone levels 1 to 4 days postpartum are very similar to levels found near estrus in jugular (Plotka *et al.*, 1967*) and ovarian (Gomes *et al.*, 1963*) venous plasma. This corroborates the findings with luteal progestins that regression of progesterone secretion occurs very quickly after parturition.

In order to study the effect of lactation on postpartum reproduction, 34 pregnant Angus cows were randomly assigned to one of three treatments. The numbers per group and treatments were 12 suckled intact (S), 13 nonsuckled intact (NS) and 9 nonsuckled mastectomized (NSM). Mastectomies were performed prior to 150 days of gestation by removing the udder and all teats. Calves were removed from the nonsuckled cows at parturition, and suckled cows were run with their calves at all times. Average quality alfalfa hay was fed at the daily rate of 13.6 kg to the S group and 6.8 kg to the NS and NSM groups.

Average intervals from parturition to first estrus for all cows in the S, NS and NSM groups were 65, 25 and 12 days, respectively ($P < .01$). No cows were detected as having "quiet" ovulations prior to the first estrus, and all cows ovulated at first estrus. Cows were artificially inseminated at each estrus until they conceived or had five services. Average values for the 6, 11 and 8 cows conceiving in the S. NS and NSM groups, respectively, were as follows: interval from parturition to conception—61, 50 and 45 days; services per conception—1.7, 2.2 and 3.0. Thus, removal of the calf at parturition shortened the interval to estrus, and mastectomy plus removal of the calf shortened the interval even further. However, the interval to conception was not shortened as much by these treatments because of the greater number of services per conception. The early heats were infertile (Short *et al.*, 1969*).

Genetic Factors

Low breeding efficiency and a high incidence of sterility were found in an inbred strain of Jersey cattle bred and maintained at the University of California at Davis. Histological examination of the reproductive system and the endocrine

organs regulating reproduction revealed abnormalities in the adrenal glands. A favorable response to exogenous cortisol suggested an abnormal function of the adrenal, and breeding of closely related animals suggested a genetic cause (Cupps, Laben and Huff, 1970*).

Subsequent analysis (table 22) showed that animals showing the defect had a low conversion of progesterone to 17-alpha-hydroxyprogesterone, 11-deoxycortisol, and cortisol; this suggests a deficiency in the hydroxylation at carbon 17 of progesterone.

Some effects of crossbreeding on reproductive performance have been summarized. Data from this study indicate breed crossing produced an over-all increase of 5.0 percentage points in the net calf crop. Breed-cross matings resulted in an increased number of cows calving and increased survival of calves from birth to weaning. Breed crossing resulted in a small (0.8 percentage points) increase in calf losses at birth. Hereford and Charolais sires, and Angus and Charolais dams, produced greater net calf crops under breed-cross mating systems. Hybrid vigor was evident in the percentage of cows calving for the Angus × Hereford reciprocal and Charolais × Hereford reciprocal matings. Hybrid vigor increased the net calf crop by 6.2, 4.4 and 2.4 percentage points in Hereford × Angus, Charolais × Hereford and Angus × Charolais reciprocal matings, respectively (Bellows, 1966*).

PHYSIOLOGY OF THE MALE

Prepuberal and puberty

Early sperm production and natural mating ability were studied in straightbred and crossbred bulls. Crossbreds reached all classification criteria at an earlier age than did straightbreds. Breed of sire differences indicated Angus-sired bulls reached all classification criteria at an earlier age than either Hereford or Charolais (Bellows *et al.*, 1964*). The pattern of seminal free amino acids appears to be related to the age of the bull. In Hereford bulls these acids are low from 4 months of age and gradually become established to the pattern of the adult bull at about 20 months of age. This is about 10 months later than the time for establishment of the adult level of fructose in semen (Hopwood and Gassner, 1962*).

Adult

With the adult male the efforts of the Regional group have been most rewarding. With the advent of artificial insemination in cattle, and successful freezing of bovine spermatozoa for use in insemination in the early 1950's, much work was conducted to find a means for assessing the prepotency of sperm. Numerous publications from the Washington and Colorado groups have dealt with this and the biochemistry of semen (Erb *et al.*, 1950*; Erb and Ehlers, 1950*; Erb and Waldo, 1953*; Erb *et al.*, 1952*, Gassner and Hill, 1952*, Ehlers *et al.*, 1953*; Flerchinger *et al.*, 1953*; Flerchinger and Erb, 1954*; Erb *et al.*, 1955*a**, *b;* Flerchinger and Erb, 1955*; Gassner and Hopwood, 1955*; Erb *et al.*, 1956*; Hopwood *et al.*, 1956*; Flerchinger *et al.*, 1956*a**, *b**; Ehlers and Erb, 1956*; Albright *et al.*, 1958*b**; Ehlers *et al.*, 1958*; Ehlers and Erb, 1958*; Erb *et al.*, 1959*; Ehlers *et al.*, 1959*; Gassner *et al.*, 1959*; Marden *et al.*, 1959*; Albright *et al.*, 1960*; Ehlers *et al.*, 1961*; Dixon *et al.*, 1961*; Masken *et al.*, 1964*; Masken *et al.*, 1965*; Cruea and Hopwood, 1966*; Masken *et al.*, 1966*, Szepesi and Hopwood, 1966*; Masken and Hopwood, 1968*). Work was also carried out by Gassner *et al.* (1952 *a*, *b*, *c**) to establish the relationships between testis and accessory sex organs in bulls.

Castration in bulls greatly diminishes ejaculate volume and the level of seminal fructose (Gassner *et al.*, 1952*a**). Seminal free amino acids also are decreased to become quantitatively but not qualitatively re-established with androgen treatment (Gassner and Hopwood, 1952*b**; Hopwood and Gassner, 1962*). Vasectomy and vasoligation reduce the levels of free amino acids of semen. The former pro-

cedure is, unexplainably, more drastic. Testosterone injection after vasectomy can increase the level of total amino acids, but glutamic acid is not restored and is similar to castration. This indicates that glutamic acid is mostly a product of the testes and epididymides. Scrotal insulation, elevating scrotal temperature by about 4° F. for a few days, renders the bull temporarily aspermic and markedly alters seminal free amino acids but does not deplete glutamic acid from the semen (Hopwood and Gassner, 1962*). The levels of seminal free amino acids appear to be correlated to the quality and breeding potential of the semen (Hopwood and Gassner, 1962*).

Effects of environmental temperature

It has been acknowledged that heat stress increases infertility. Bull semen used for artificial insemination has been suspected as a factor in lowering seasonal fertility in dairy cattle in hot climates. The influence of ambient temperature on testicular function has been the subject of numerous investigations (Moore, 1924, Phillips and McKenzie, 1934; Lagerlof, 1934; Meshaks, 1953; Gassner, 1955*). Generally, increasing testicular temperature causes a cessation of spermatogenesis with a simultaneous rise in the initial fructose content of the semen. Initial motility, sperm density, and total sperm count have been used to show the adverse effects of temperature on spermatogenesis. It should be remembered that experiments with bulls have been done with artificial means of raising the ambient temperature, and not by studying semen under normal environmental changes. The study reported below was designed to determine the influence of Arizona summer temperatures upon the fructose level, initial motility, and sperm density of semen from bulls maintained under normal field conditions.

Eight Holstein bulls located at three commercial dairies in central Arizona were used in the study. Semen was collected from each bull twice weekly for artificial insemination. From these collections, samples were obtained at biweekly intervals from May 2 to November 26 for laboratory analysis. Immediately after collection, motility was determined and the sperm killed to prevent fructolysis. Motlity was rated from 0 to 5.

The average value for each of the three variables (sperm density, fructose, and motility) for each sampling date was compared with the mean wet-and-dry-bulb temperatures of the day preceding each respective sample. Although an analysis of variance showed a significant difference among dates in both fructose and sperm concentration; Duncan's multiple range test indicates that this was not due to any seasonal trends. Furthermore, no relationship can be seen between temperature and fructose level. Motility, however, seemed to be temporarily depressed when climatic temperatures suddenly soared to abnormal heights (from 111° up to 118°F.) in mid-July. Motility ratings ranged from zero to four with an average of only two. By the next sampling date motility had returned to normal. Apparently the extreme temperatures had an acute but temporary effect on spermatogenesis. Oddly enough, a depression in sperm density, and to some extent motility, occurred in September and October after climatic temperatures had moderated.

Although fertility of bulls is apparently not significantly affected by normal Arizona summer temperatures, high ambient temperatures may have a cumulative effect upon bulls, with consequent reduction of semen quality only after a period of time (Stott and Williams, 1962*).

Effects of exogenous hormones

The influence of androgens, progestins and estrogens on the reproductive system of bulls has been investigated. Estrogen (estradiol-17β-benzoate) given to bulls in large doses over a long period of time damages the spermatogenic system without causing total aspermia, while testosterone administered as the propionate has little effect. Loss of seminal fructose together with histological changes show that the Leydig cell system is the last to be affected (Gassner, 1952*; Gassner *et al.*, 1952*a**). Estradiol admin-

istered at levels below 2 mg per day (calculated on the basis of 3 injections per week) had little effect on the semen from normal bulls, but at 5 to 7 mg per day it lowered the quality of the semen produced (Cupps *et al.*, 1960*). Volume of ejaculate, concentration of fructose and citric acid, and motility were reduced and the percentage of abnormal sperm was increased. A high proportion of the abnormal sperm had either looped or bent tails. Supplementary testosterone given simultaneously with exogenous estrogen did not prevent the increased incidence of abnormal sperm (Cupps and Briggs, 1965*). Changes in the function of the epididymis appear to be related to the formation of large numbers of abnormal sperm.

Exogenous testosterone at 200 mg per day partially inhibited spermatogenesis and increased production of sperm with abnormal heads, midpieces, and tails, but also caused a rise in fructose and citric acid in the semen (Richkind *et al.*, 1967*). Progesterone at 100 or 200 mg per day caused a decrease in the concentrations of fructose and citric acid and the motility and volume of ejaculate. Spermatogenesis was abnormal and partially suppressed. The number of abnormal sperm in the semen was increased, and tailless sperms were the most common type of abnormality (Matsuyama *et al.*, 1967*).

Effects of testicular biopsy

In the evaluation of the reproductive performance or potential of bulls, examination of the semen has been of value; however, it was thought that examination of the testis by histological means through biopsy would be of paramount importance. Biopsy of the bovine testicle was studied (Hill and Gassner, 1955*, Gassner and Hill, 1955*) and found to be accompanied by severe functional damage to testicular tissue. Unlike that of other animals, the testis of the bull is extremely vascular. The extent of its vascularity was determined by angiography (Gassner, 1955*) and the type of damage assessed to be similar to that caused by hyperthermia. Semen analyses following biopsy showed the presence of spherical cells of various sizes which were heavily granulated, and often showed multinucleated resembling giant cells; in normal semen these are absent or are found infrequently. After biopsy they appear in noticable numbers about a week after surgery; they become progressively more numerous as semen quality becomes poorer and, possibly, remain for several months until semen quality is improved. These cells were found in ejaculates of every bull subjected to steroid therapy and thermal insulation, and after every biopsy. It is evident that the appearance of large numbers of these cells in semen samples is pathognomonic of testis degeneration involving primary atrophic changes in the seminiferous tubules. This is in agreement with Lagerlof (1934) and Blom (1950) who observed similar cell forms.

Effects of ionizing irradiation

Another influence on spermatogenesis and spermatozoa which was studied by investigators in the Regional Project was the effect of ionizing irradiation. Wu and Prince (1963*a**) showed that irradiation of rabbit spermatozoa up to 10,000 rad of x-irradiation caused no noticeable effect on oxygen uptake or survival. Irradiation at doses that greatly depress motility may not affect respiration. Even at higher intensities (100,000 rad, 320,000 rad or 640,000 rad of gamma irradiation from Co-60) impairment of respiration was not immediately apparent. Effect of irradiation on sperm DNA in terms of relative absorbance of DNA-Feulgen complex was not detectable at 30,000 rad of gamma irradiation. At 320,000 rad, however, the complex of the irradiated cells was significantly affected. Rabbit spermatozoa, even with their richly endowed seminal catalase, are more sensitive (with respect to sperm motility) to ionizing irradiation than are spermatozoa of bull or ram.

Bulls testes were irradiated with doses of x-rays between 50 and 40,800 rad (Gillette *et al.*, 1963), and abnormal sperm were seen as early as 2 weeks afterwards. Decreases in sperm numbers were seen as early as 7 weeks after exposure to 800 rad, with longer times re-

quired to observe changes in sperm from bulls exposed to higher levels. The physical score (Carrol *et al.*, 1963) of semen decreased 5 weeks after exposure to 800 and 400 rad, and after a longer time for lower doses. Radiation depressed the rate of fructolysis and oxygen uptake by sperm of bulls at all exposure levels. An increase in the initial amount of fructose in semen was found to correlate with decreases in sperm numbers; this also occurred in bulls subjected to testicular hyperthermia (Gassner, 1955*). Recovery after irradiation took between 30 and 40 weeks. Sperm concentration returned to only 65 per cent of pre-irradiation values at 40 weeks after exposure to 800 rad, and this level was maintained until termination of the study 52 weeks after exposure.

Morphological studies

Electron microscopy of bovine spermatozoa has been extensively investigated by Wu and associates (1954*, 1955*, 1963*b**, 1965*, 1966*a**, *b**, 1967*, 1968*a**, *b**). Spermatozoa from bovine epaculates and epididymis were studied. Epididymal spermatozoa were fixed *in situ* in short lengths of tubules dissected from the cauda epididymis, or were fixed in suspensions as for ejaculated sperm after expressing the spermatozoa through incisions in the tubule wall. Fixation was carried out for 1 hour in 1 per cent osmium tetroxide (Palade, 1952) with or without prefixation by glutaraldehyde. Fixed specimens were dehydrated with methanol and propylene oxide, stained with uranyl acetate, and then embedded in Epon (Luft, 1961).

Plate I, figure 1, shows various planes of sections of bovine spermatozoa observed in an electron microscopic field. Without ultra-thin sectioning technique the structures of spermatozoa cannot be clarified even with the electron microscope (plate I, figure 2). The acrosome is a flattened cap-like structure bounded on either side by a single continuous membrane (plate I, figure 3). The cap may extend considerably beyond the most anterior margin. Sometimes this acrosomal extension is greatly enlarged and folded back upon itself (plate I, figure 4).

Posteriorly, the nucleus is enveloped in a postnuclear cap which is comprised of a dense homogenous layer intimately applied to the nuclear membrane. At its most anterior limit the postnuclear cap slightly overlaps the posterior margin of the acrosome. Sections of the nucleus, oblique to its longitudinal axis but normal to its flattened surfaces, often exhibit regularly spaced annuli within the electron dense matrix of the postnuclear cap (plate I, figure 5).

At its posterior pole the spermatozoan nucleus is indented, and in the concavity so formed lies the proximal centriole (plate II, figures 6 through 9). This centriole appears as a "hollow" cylindrical structure. It is oriented with its long axis in the plane of the flattened surface of the nuclues and oblique to the longitudinal axis of the cell. In transverse sections this centriole appears to consist of a thick electron-dense wall and a less dense center. Embedded in the wall are 27 tubules arranged in 9 rows of 3 tubules each. Each of these 9 tubular arrays is oriented at an angle of approximately 40 degrees to a line tangential to the circumference of the centriole. This arrangement of 9 rows of tubules gives the cross-sectional view of this centriole a "pin-wheel" appearance similar to that described in cilia and flagella by Gibbons and Grimstone (1960). The periphery of the wall is scalloped in conformity with the distribution of the underlying tubular arrays.

The principal organelle in the neck region of bovine spermatozoa is the connecting piece composed of cross-striated columns. The two major columns or implantation plates (plate II, figures 6 and 7) are fused at their anterior ends to the basal nuclear indentation. Minor columns have been observed in some sections and there are probably seven of them. The micrographs also show distinct lines between the columns and their adjacent coarse fibrils (plate II, figure 7).

The mitochondrial helix, the characteristic feature of the middle-piece, is comprised of individual mitochondria which form a helical structure around the axial filament (plate II, figures 10 and 11). At the most anterior end of the middle-piece, mitochondria are arranged in a number

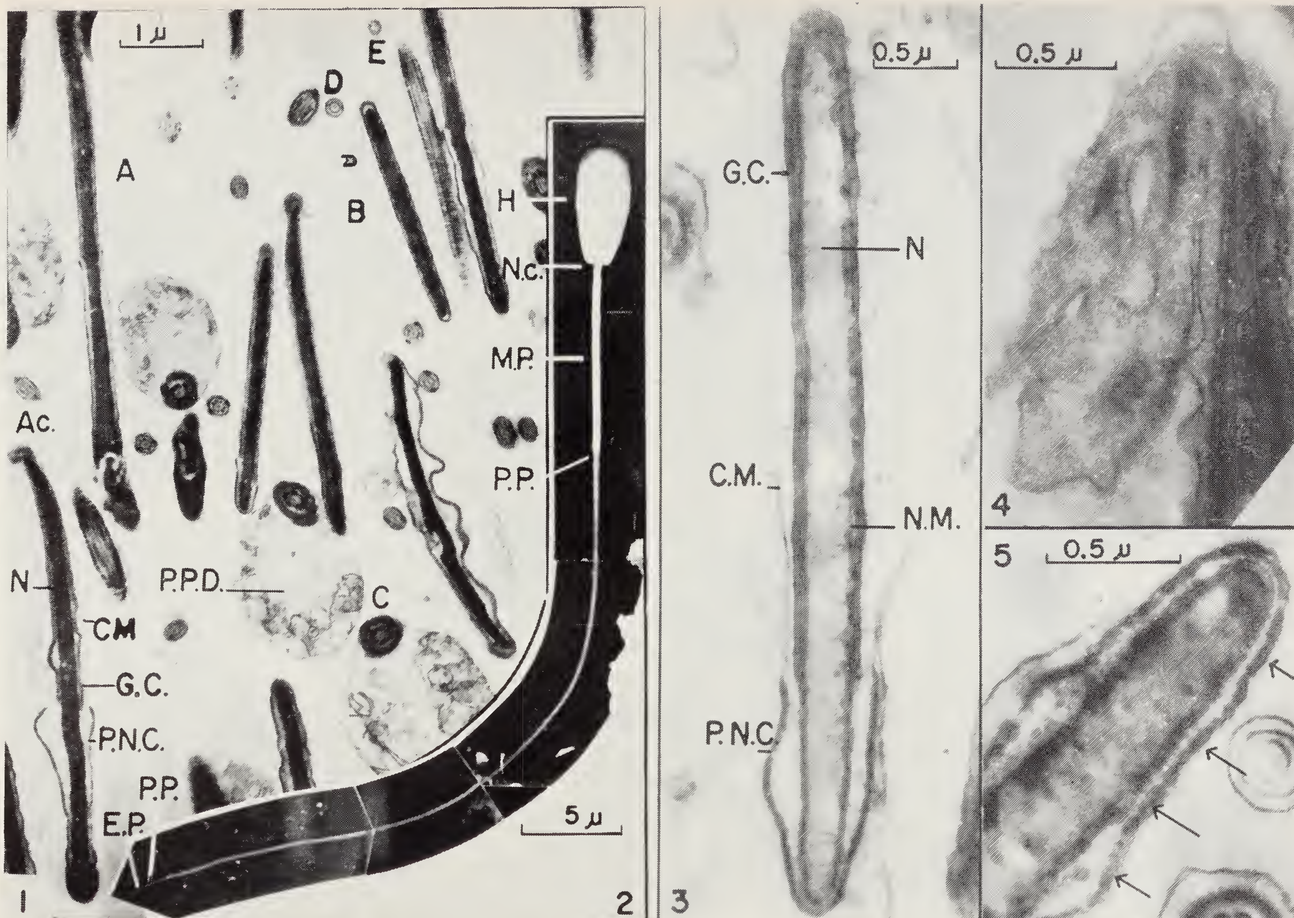


Plate I. Electron micrographs showing various planes of section (1) and diagrammatic representation of a bovine spermatozoon (2). Also sagittal sections through the sperm head (3) and acrosome (4) and oblique section of the nucleus. (5).

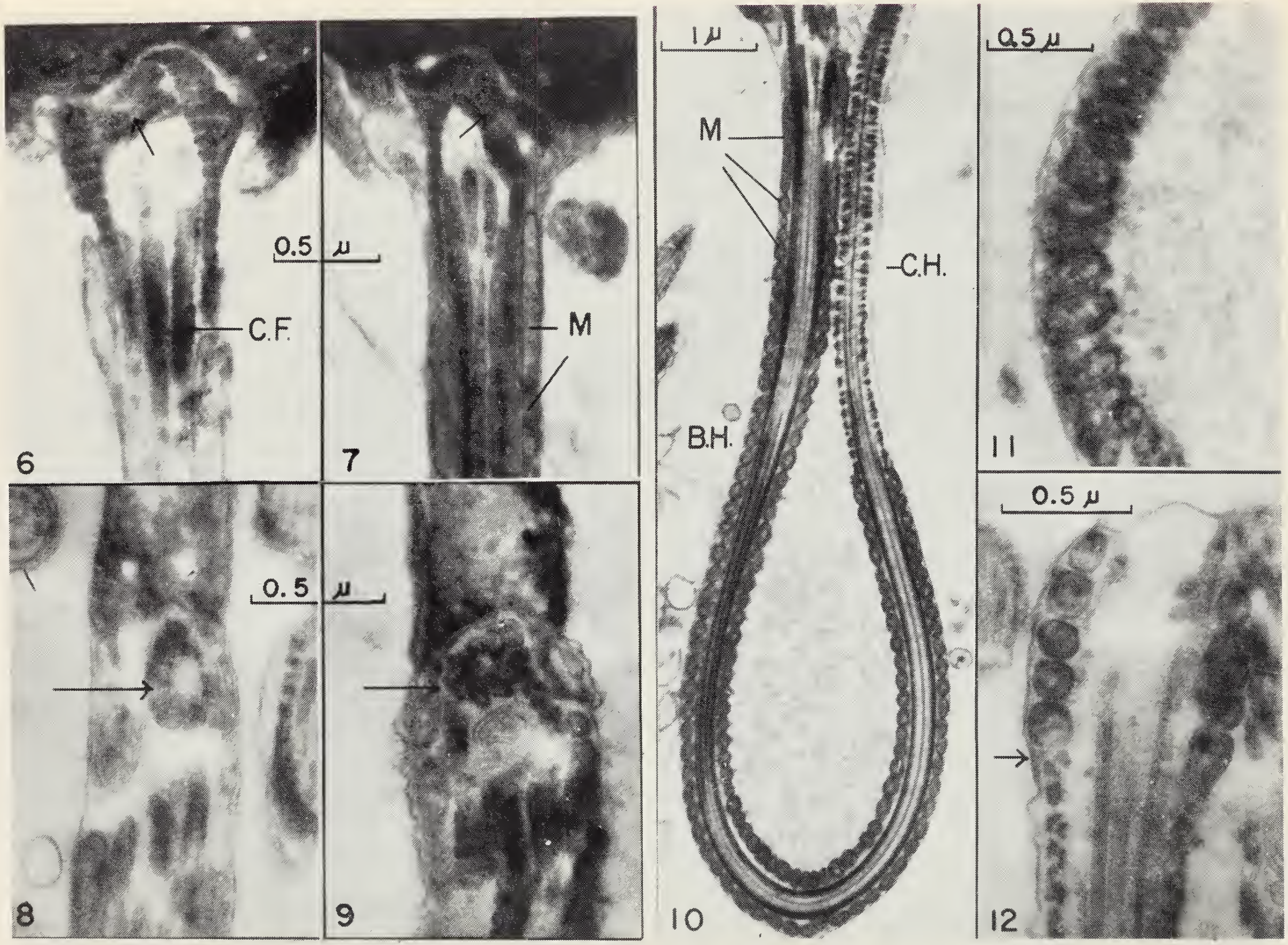


Plate II. Frontal sections through the neck and middle piece (6, 7) and sagittal sections of head-neck junction (8, 9) showing the structure of proximal centriole. Also longitudinal sections of middle-piece (10, 11, 12) showing the arrangement of mitochondria.

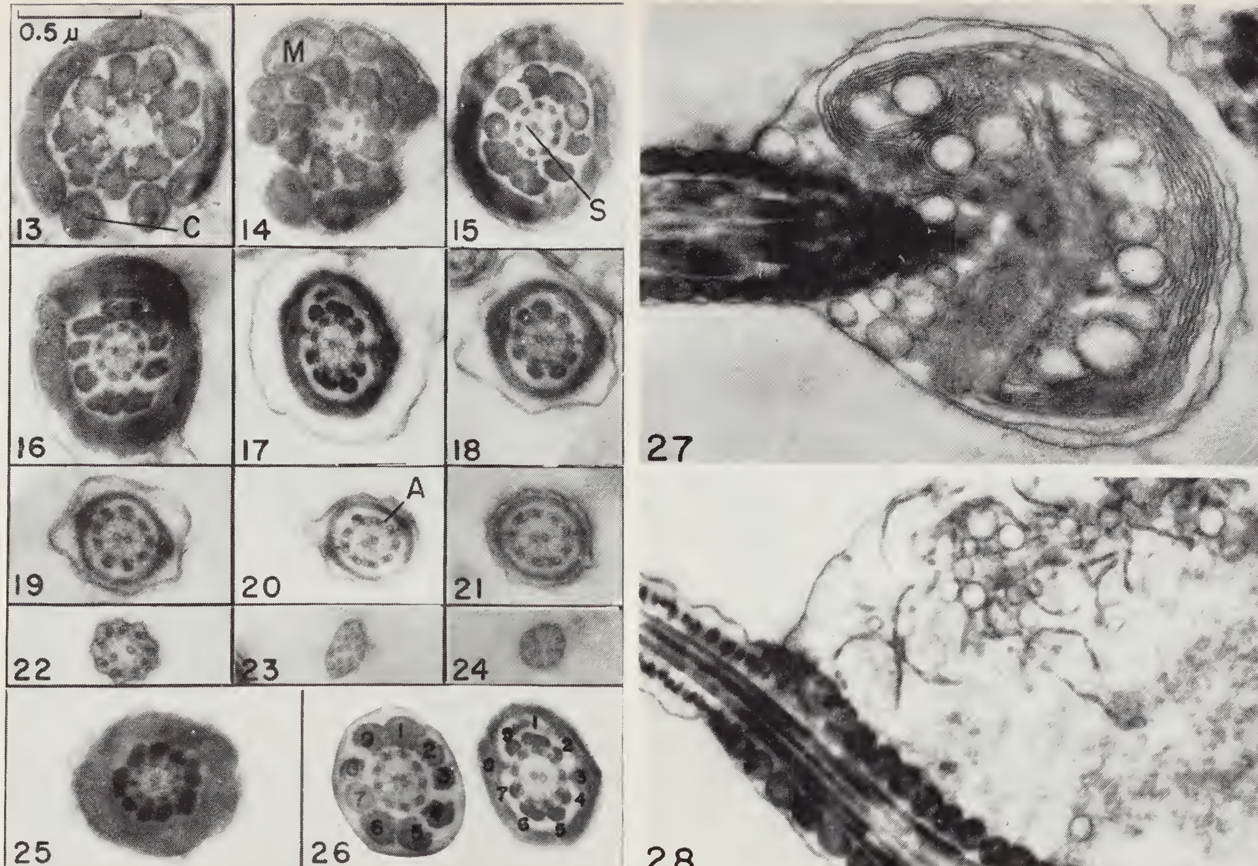


Plate III. Transverse sections of axial filament complex (13–26) showing its "9 + 9 + 2" pattern at the middle-piece and the diminishing size of the 9 coarse fibrils as they pass from the anterior to the posterior region of the tail. Figure 26 shows fibrils by number. Figures 27 and 28 show the structure of the cytoplasmic droplets. C (fig. 13) = Thickened fibrous sheath; M (fig. 14) = mitochondrian; S (fig. 15) = electron-dense material connecting fibrils; A (fig. 20) = modification of fibrils toward tip of tail.

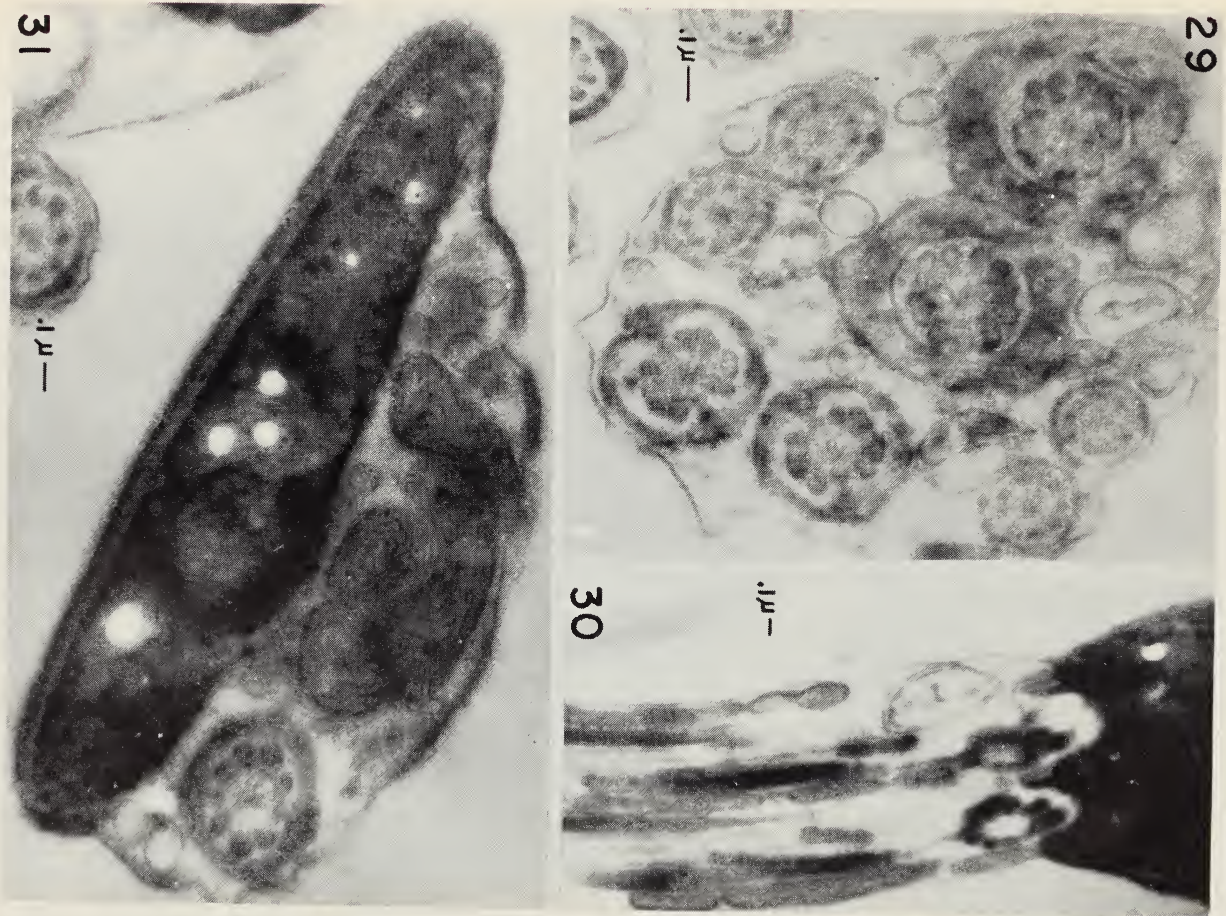
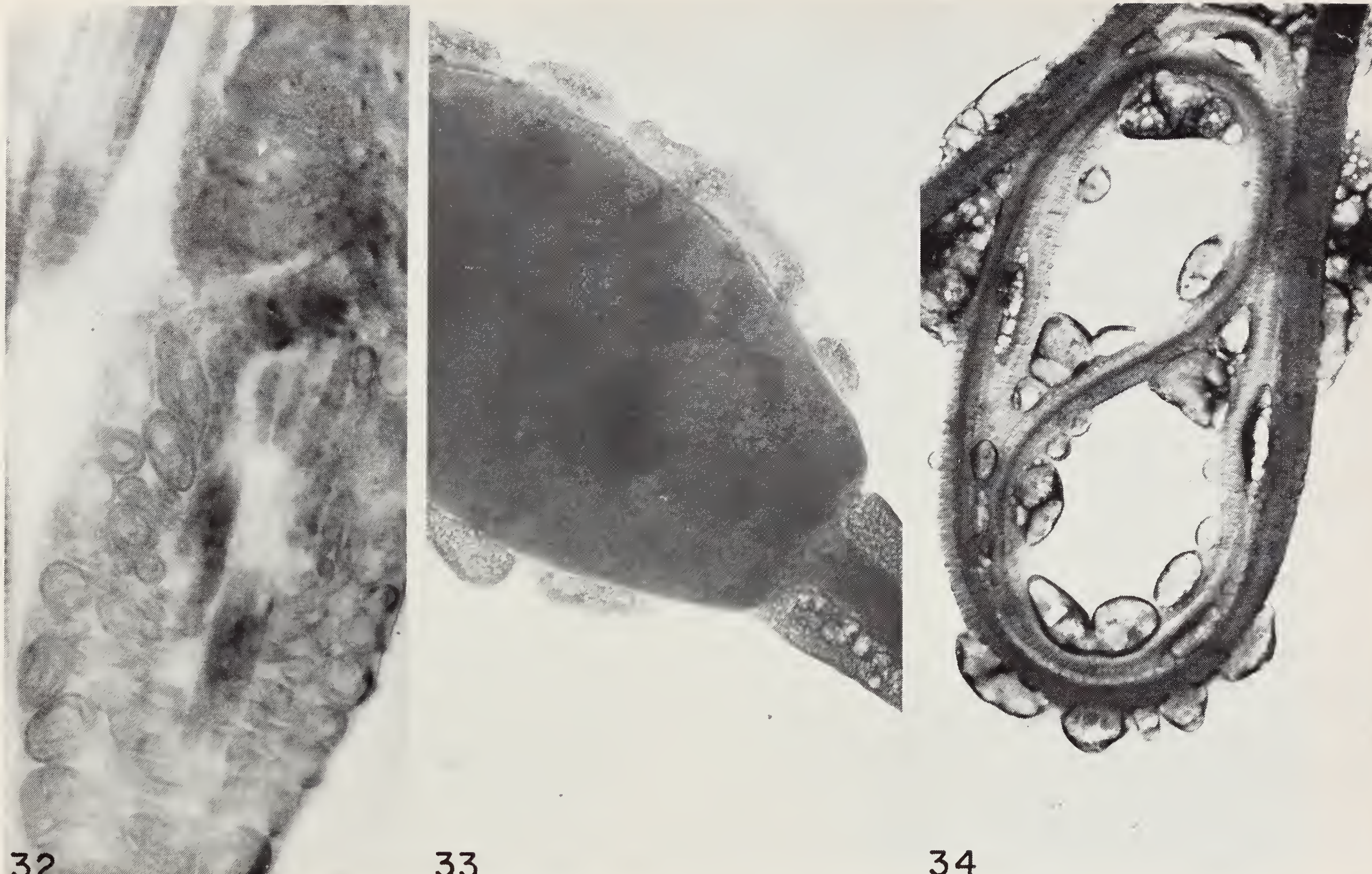


Plate IV. Sectional views of some bizarre forms of spermatozoa with several tail sections included in a single cytoplasmic droplet (29); double tail (30) and section of nucleus with tail structures discernible in lifted nuclear membrane (31).



32 33 34

Plate V. Disorderly arrayed mitochondria at the neck-tail junction of a spermatozoon from an infertile bull (32), and structures of an amorphous nature which appear along the free surface of spermatozoa subjected to mechanical stress (33, 34).

of longitudinally-oriented rows, Further along the middle-piece the rows of mitochondria become wound into a tight helical structure.

The transition from the middle-piece to the principal piece is marked by the termination of the mitochondrial helix, the presence of the annulus, and the commencement of the fibrous sheath (plate II, figures 10 and 12). The annulus appears in longitudinal sections as a wedge of electron-dense material closely applied to the cellular surface, with its broad base in contact with the final turn of the mitochondrial helix. The fibrous sheath appears in cross sections of the tail as an electron-dense band enclosing the axial structure. Thickenings of this band are located in the plane that passes medially through the two central fibrils opposite the coarse fibrils numbered 3 and 8 (plate III, figure 26, shows numbered fibrils). Longitudinal sections suggest that the bands are composed of dense material which has an almost square appearance in median sections of the tail structure (plate III, figures 13 and 15). These bands appear to be connected to one another along the longitudinal axis by coarse fibrils continuous with the thickened regions of the bands.

The axial filament core consists of two central fibrils surrounded by a ring of 9 doublets (plate III, figure 20). This core persists into the end-piece with some modifications of its fibrils toward the tip of the tail (plate III, figures 20 through 24). In the middle-piece (plate III, figures 13 through 16) and extending to the principal-piece (figures 17 through 21), an additional ring of 9 coarse fibrils is found to encircle the axial filament core. These 20 fibrils are embedded in the homogenous interfibrilar matrix. They are enclosed either by the mitochondrial helix in the middle-piece or by the fibrous sheath in the principal piece (plate II, figure 10).

The central pair of fibrils are tubular in appearance (plate III, figures 13 through 21). They appear to be connected by poorly defined arcs. In transverse sections, "spokes" are frequently observed to radiate from the paired central fibrils toward each of the nine fibril doublets. An electron-dense thickening may be seen near the center of each spoke. One member of each of the nine doublets has an annular profile similar to that of the central pair, while the other appears uniformly dense. Each of the electron-dense members carries a pair of short projections on the surface distal to its partner fibril.

The individual coarse fibrils comprising the outer fibrillar ring are seen to be much larger than the individual doublets (plate III, figures 13 through 16). In some transverse sections, the nine coarse fibrils may be of similar size or shape. However, in sections farther from the head, three or sometimes four of these nine coarse fibrils are distinctly larger than the others. The cross-sectional views of some may appear as elongated "dumbbell-shaped" structures. For identification (as shown in plate III, figure 26), one may follow the usual convention of Bradfield (1955), Fawcett (1958) or Gibbons (1961). Farther posteriorly, the coarse fibrils diminish in size and terminate at various levels. First, the fibrils numbered 3 and 8 are much reduced in size. Then 4 and 7, 2, 9, 5, and 6, and finally 1 disappear in that order (plate III, figures 17 through 21). Toward the end of the principal piece the axial filament consists only of the central pair and the nine doublets. In most of the micrographs the reduced fibrils numbered 3 and 8 of the outer coarse fibrils seems to persist as distinct, although small, dense structures after the termination of the other 7 coarse fibrils.

Termination of the fibrous sheath marks the anterior limit of the end piece (plate III, figure 22). Here, this region possesses only the usual axial core of the $9 + 2$ pattern, and the spoke-like arrangement seems to connect the nine doublet fibrils with the central pair in the more anterior tail segments no longer identifiable. Toward the posterior end of this terminal tail segment the doublet character of the peripheral filament is lost, and only annular profiles lacking projecting arms appear in a rather haphazard arrangement (plate III, figures 23 and 24). As many as thirteen single profiles have been observed in one such section (plate III, figure 24). Still farther pos-

teriorly, the number of these single tubular structures is reduced until finally none remain. Throughout the end-piece the filamentous structures are embedded in a matrix material of low electron-density which, in turn, is enclosed within the cellular membrane.

Protoplasmic droplets of bovine spermatozoa appeared to consist of numerous vacuoles and concentric spheres of parallel membranes. Droplets which resemble the Golgi complex consisting of a cluster of parallel membranes were also observed (plate III, figures 27 and 28). The protoplasmic droplets attached on the spermatozoan flagellum have long been regarded as cellular remnants during spermatogenesis. They usually peel off and disappear in the majority of sperm cells found in normal ejaculates. However, the morphological complexity of the droplet as revealed in thin section with the electron microscope seems to indicate that this sperm organelle may play some important role, probably of secretory function, in spermatozoa.

Abnormalities in the sperm acrosome and nucleus have been described in mice with hereditary sterility, and in many cases of bovine infertility. Acrosome abnormalities have also been reported in spermatozoa from boars with breeding difficulties. Coubrough and Barker (1964) reported a specific midpiece abnormality in bovine spermatozoa associated with sterility. In the present study, similar types of spermatozoan abnormalities in a sterile bull where the mitochondria seemed to be disorderly arranged have been noted (plate V, figure 32). Because this abnormality cannot be observed with a conventional light microscope, the fine structure of sperm as revealed by the electron microscope could be used to identify certain types of infertility. Electron micrographs of other bizarre forms of spermatozoa have also been obtained in this study. These include tail, head, or nuclear abnormalities (plate IV, figures 29 through 31).

Variation in the freezability of sperm cells from different individuals or species have been observed in the preservation of spermatozoa by low-temperature freezing technique. Among other factors the difference in cellular membrane ultrastructure may attribute to the success or failure of semen preservation. One phase of this study was, therefore, the search for techniques for examining the ultrastructure of spermatozoan membrane. Preliminary studies of sperm membrane with freeze-etching techniques were made, and studies were also made to isolate the sperm membrane, including the use of lysozyme. It was noted that after subjecting the sperm to mechanical stress, structures assuming elongated profiles of an amorphous nature closely applied along the free surface of the sperm head and flagellum were observed (plate V, figures 33 and 34). The nature and significance of these structures are under investigation.

Seminal fluid

Contributions of various portions of the reproductive tracts of bulls to the seminal fluid have been measured to obtain further understanding of the process of ejaculation, and to assist in the evaluation of seminal fluid for use in insemination. Fractionation of the ejaculate is possible in the boar, dog, stallion, and man but is not possible in the bull when conventional methods of collection are used. Electroejaculation may be used for this purpose in the bovine, but this yields semen having chemical and physical properties different from those collected with the artificial vagina (Faulkner *et al.*, 1964*; Mann, 1964). Another approach to assessing contribution of the accessory sex organs to the ejaculate is vasectomy (Gassner and Hopwood, 1952*; Hopwood and Gassner, 1962*; Aslbers, 1966). removal of the seminal vesicles (Hess *et al.*, 1960; Shah *et al.*, 1968; King and Macpherson, 1969) or use of animals with congential aberrations in accessory glands (Aalbers, 1966). It is apparent that the seminal vesicles contribution to the volume of the sperm-rich fraction of the ejaculate, to lowering the pH of this fraction, and to the morphology of the spermatozoa, but they do not affect the fertilizing capacity of the spermatozoa, at least in natural service (Faulkner *et al.*, 1968). It appears that the pre-sperm fraction of the ejaculate is from the prostrate,

disseminate prostrate, and bulbourethral glands as the bull lacks urethal glands (Kainer *et al.*, 1969). The seminal vesicles contribute fructose, the epididymal contribution is characterized by sperm and glycerlphosphorylcholine, and the remaining areas are marked by the delivery of chloride to the ejaculate (Seidel and Foote, 1970).

Steroid metabolism

Lindner and Mann (1960) and Lindner (1961) demonstrated that the bull testicle produces testosterone and androstenedione, and they found the levels to be elevated when human chorionic gonadotropin (HCG) was administered. Savard *et al.* (1961) determined the output of testosterone and androstenedione from the HCG stimulated testicle. Martin (1964) reported on the collection and analysis of spermatic venous blood as well as jugular blood before as well as after HCG (20,000 units per day) administered over 6 days. The whole blood averaged 1300 μg testosterone per liter and 175 μg per liter for androstenedione. The ratio of testosterone:androstenedione was 7:1, in agreement with Savard *et al.* (1961). Lindner (1961) had reported a ratio of 15:1 and found androstenedione to be higher in the prepubertal animal.

Although estrone was isolated from the urine of the steer and the bull (Marker, 1939), and a positive color reaction for phenols (Kober) was obtained for the bovine testicle (Velle, 1958*a*), it is not assured that the bovine testicle is responsible for the estrogen in blood. Martin (1964) detected similar amounts of estrone and estradiol in blood removed from the jugular vein of a bull, as compared with blood obtained from cannulation of the testicular vein. Possibly the testicle produces but does not secrete estrogens while the circulating estrogen may be a product of the adrenal gland. Cannulation of adrenal veins of three young bulls with indwelling catheters was carried out, and blood was collected before and after ACTH stimulation. Corticotropin administration elevated the corticoid level in blood as expected but lowered the estrogen output of the adrenal. A special procedure to detect and identify estogens (estrone and 17ß-estradiol) for this study was devised employing labeling of the blood-borne estrogens with ^{14}C-labeled dimethyl sulfate (Pearson, 1965*).

Pearson and Martin (1966*) injected 17ß-estradiol-4-^{14}C into a bull and plotted disappearance of radioactivity from the blood to obtain two discrete half-lives of 4.5 and 15 minutes, thus indicating enterohepatic recirculation of the steroid. Radioactivity in bile reached a maximum concentration at 12 minutes. In 3 hours, 53 per cent of the dose was excreted in bile and 4 per cent in urine. The material in bile was all conjugated and hydrolyzable with β-glucuronidase. Estradiol-17α and estrone accounted for 94 per cent of the radioactivity in bile; the remaining 6 per cent was more polar than estriol and was not identified.

A crossbred steer was injected subcutaneously with testosterone-4-^{14}C (120 mg, 385 μC). In 24 hours 15 mg of the testosterone was execreted in the feces, none of which was conjugated. Two of at least six steroid metabolities were identified as epitestosterone-^{14}C (37 per cent of the ^{14}C excreted) and 17α-hydroxy-5β androstan-3-one (17 per cent of the ^{14}C excreted) (Martin, 1966*). Interest in the adrenal cortex has led to study of the secretion of this gland. Injection of 4-^{14}C-cortisol revealed that about 95 per cent of the injected dose was secreted in bile and about 80 per cent of this was excreted in the feces. Preliminary separations and partial purification revealed most of the cortisol to be degraded to 19 carbon steroids in which the ketone groups were reduced to hydroxyl groups. The potential ability of the adrenal to convert precursors was therefore evaluated, because extensive fecal changes prevented quantitative measurement (Cupps *et al.*, 1964*). The conversion of progesterone and pregnenolone by adrenal homogenates shows that animals showing a decreased growth rate, lowered fertility and a delayed development of secondary sex characters to be lower in cortisol synthesis (Cupps *et al.*, 1970*).

With respect to the adrenal products in dairy cattle, Estergreen and Venkataseshu (1967*) positively identified corticos-

terone and cortisol in jugular plasma of cows with a mean cortisol:corticosterone ratio of 2.40:1. Bulls with nodules in the fascicular zone of the adrenal secrete abnormally large amounts of cortisol, and produce semen with rather specific characteristics (Cupps, *et al.*, 1959*). There is a high concentration of spermatozoa accompanied by a decrease in fructose and citric acid concentration, and in volume of ejaculate. In the early stages of the condition the motility is high and abnormal spermatozoa are intermediate in number. As the condition progresses, motility decreases and abnormal spermatozoa increase. In most cases studied, concentration of sperm remains high and spermatogenesis remains normal. Exogenous cortisol or cortisone produces similar effects in normal bulls (Cupps *et al.*, 1960*a**) except that the decrease in motility found in some of the affected bulls was not apparent in bulls receiving exogenous cortisol. This difference is probably due to the short period over which the injections were given. Supplementary testosterone does not increase the concentration of seminal fructose in animals with this condtion, and further experiments are needed to determine the mechanisms involved. Incubations of the adrenal cortex from two of these animals indicate that they produce more than four times as much cortisol as do normal bulls of similar breeding (Cupps *et al.*, 1964*a**).

In old bulls having extensive degeneration of the testes, the glomerular zone of the adrenal cortex is hypertrophied (Cupps *et al.*, 1959*). In contrast to the testicular hypoplasia found in young bulls, the testes in these animals are of normal size. The extent of the tubular degeneration varied in individual animals; some animals showed limited lesions, and others showed complete destruction of the seminiferous epithelium. The degree of tubular degeneration is reflected in the extent to which spermatozoa concentration in the semen is depressed. When the damage to the testis is limited, concentration is depressed only slightly, but the degeneration may be so extensive that the semen is aspermic (Cupps *et al.*, 1964*b**). In contrast to the hypoplasia found in young bulls, degeneration occurs at early stages of spermatogenesis, probably in the spermatogonia, and once the cells are past this stage they develop normally (Cupps and Laben, 1960*b**). When degeneration is extensive, all the spermatogenic cells are destroyed and only the sertoli cells lining the tubule are left. When the testicular damage is extensive, the kidneys are also damaged (Cupps *et al.*, 1964*b**) and the bulls have polyuria that is resistant to antidiuretic hormone. A similar polyuria can be produced in normal bulls with excessive exogenous deoxycorticosterone, thus indicating that these animals are secreting excessive amounts of the salt regulating steriods. Whether excessive salt-retaining steroids are causing the damage to the testis is not known, but available information suggests that damage to the kidney causes excessive secretion of steroids and that damage to the testicular epithelium results from vascular damage within the testis.

SUMMARY

Experiments conducted by the cooperating experiment stations of the western region have materially increased existing knowledge of normal reproductive processes in the male and female cattle and have in part ascertained the changes associated with impaired fertility.

Based on data resulting from these experiments, the estrous cycle may be visualized as follows. On the 16th to the 18th day of the cycle a temporary depression of progesterone secretion by the corpus luteum occurs; this is accompanied by an accumulation of secretory granules in the basophil cells of the pituitary, a slight increase in the secretion of blood levels of gonadotropins, and a rising secretion of estrogen. About 24 hours before the estrus the progesterone secretion falls abruptly, and the rising titer of gonadotropin stimulates further growth of the intermediate-sized follicle. As the titer of estrogen rises the animal comes into estrus and large quantities of luteinizing

hormone are released abruptly. Ovulation follows this release in about 24 hours. The granulosa cells begin to luteinize and the corpus luteum begins to form. Cells from the theca interna appear to revert to a resting stage for a variable length of time (up to 8 days) and then begin to luteinize. The corpus luteum grows rapidly and reaches its mature size by the 12th to 15 day of the cycle, and the rate of secretion of progesterone increases in parallel with luteal size. As the corpus luteum grows, one or two of the small tertiary follicles also grow and reach an intermediate size by the 6th to 8th day of the cycle. Quite frequently a temporary minor increase in estrogen secretion, probably within a 24-hour period, occurs one or more times from 4 to 14 days of the cycle depending on the individual. Thus, two peaks of estrogen secretion occur in the normal cycle, a major one during late proestrus and early estrus and a minor one during diestrus. In some animals with impaired fertility, the secretion of estrogen is not so well regulated, and may show several fairly large peaks during the cycle. The physiological mechanisms controlling estrogen secretion, and the source of the estrogen secretion during the diestrous phase of the cycle, are still unknown and attempts to correlate them with pituitary gonadotropins (particularly with FSH) have not been successful. Continued excretion of estrogen following ovariectomy indicates an extrovarian source—perhaps the adrenal cortex.

The quantitative and qualitative features of the biosynthesis, secretion, metabolism, and excretion of the major steroid hormones and their metabolites have been evaluated in detail. Three major estrogens have been identified. Progesterone and closely related compounds and their metabolites have been measured in tissues, urine, and recently in the peripheral blood. Changes in the amounts and differences in secretion patterns during the cycle, pregnancy, parturition, and postpartum period have been measured and idetnified. The formation of testosterone and closely related steroids has been studied in the male during various reproductive stages. The major adrenal cortical steroids have been separated, and some progress has been accomplished in measuring their synthesis, metabolism, and blood concentrations.

Compounds with gonadotropic activity have been found in the placenta and endometrium.

The effects of exogenous steroid and gonadotropic hormones on the reproductive organs and processes in the female and male have been studied extensively. In the female, exogenous estrogen at low levels will maintain the corpus luteum, cause failure of implantation, and modify the estrous cycle and regulate the release of gonadotropins. At intermediate levels, damage to the follicles of the ovary occurs and the endometrium is modified. At high levels the behavior pattern of the cow is affected, and the ovaries and gonadotropin secretion are depressed. Estrogens depress spermatogenesis in the male, modify the spermatozoa, change the chemical characteristics of the seminal plasma, and have a prolonged inhibitory effect on the reproductive system. Progesterone and closely related synthetic progestins will maintain pregnancy in the castrate female. Parturition is not entirely normal under these circumstances because the placenta is usually retained. Progestins are effective agents for the synchronization of the estrous cycle, and in combination with estrogen they may modify the post partum interval; they also modify spermatogenesis and the constituents of the seminar plasma in the male. Exogenous androgens depress spermatogenesis in the male, and following castration they partially restore many of the compounds found in normal semen. Exogenous gonadotropins (particularly FSH) modify follicular patterns in the ovary, and they show some promise for use in the experimental production of a higher incidence of multiple births in cattle.

Sperm production and mating in straight and cross-bred adolescent bulls have shown that Angus-sired bulls reach puberty earlier than those sired by Hereford or Charolais. Seminal-free amino acids are low in Hereford bulls reaching a mature level at about 20 months of age. The adult level of fructose is established

at about 10 months of age. The ultrastructural morphology of normal and abnormal spermatozoa have been described. Data from experiments and field trials have established important criteria for estimating the relationship between the constituents of the semen and the morphological and metabolic characteristics of spermatozoa and their potential fertility.

Data obtained from experiments conducted in this regional project have increased our knowledge about several conditions which cause impaired breeding efficiency. Histological criteria and changes in cyclic characteristics of the estrous cycle have established the fact that several types of ovarian cysts lower fertility in affected animals. High environmental temperatures lower the fertility in the female, particularily those producing heavily, and evidence suggests that abnormal ovarium and adrenal function are the primary factors involved. Hypersecretion of the glucocorticoids and the salt-regulating steroids, and hyposecretion of glucocorticoids in response to heat stress or because of genetic defects, lower fertility in both female and male. Although the above factors do not explain all of the conditions which result in sterility or lowered fertility in cattle, they represent conditions which were unknown before the research was undertaken. Improved methods for measuring physiological parameters associated with normal reproduction and lowered fertility as reported here will prove to be most valuable in further improving the control of reproduction—and this in turn will improve the efficiency of meat and milk production in our domestic animals.

TABLES 1–22

Tables 1 through 12 and 15 through 17 were prepared by W. C. Foote and co-workers.
Tables 13 and 14 were prepared by R. E. Erb and associates.
Tables 18 through 21 were prepared by W. D. Foote and co-workers.
Table 22 was prepared by P. T. Cupps and co-workers.

TABLE 1. THE MEAN EFFECT OF 17β-ESTRADIOL AND PROGESTERONE ON OVULATION, CL MAINTENANCE AND FOLLICULAR DEVELOPMENT. (Piper and W. C. Foote, 1968).

Treatment[1]	Ewes with estrogen-induced CL (%)	Maintenance of natural and induced CL[3] 24th day of cycle (%)	Follicular development at day 16 of treatment cycle; ewes with at least one follicle > 6 mm (%)
Control	0[a2]	0[a]	80[a]
Estradiol	77[b]	8.3[a]	53[a]
Estradiol + Estradiol daily	67[b]	50.0[b]	0[b]
Estradiol + progesterone daily	20[a]	0[a]	80[a]

[1] Initial 2 mg estradiol on day 4. Daily estradiol was 1 mg from day 5 to 24. Daily progesterone was 5 mg from day 5 to 24.
[2] Values in the same column not bearing the same superscript differ significantly ($P<0.05$)
[3] The patterns of regression were similar for natural and induced CL.

TABLE 2. THE MEAN EFFECT OF 17β-ESTRADIOL ON OVULATION, CL MAINTENANCE AND FOLLICULAR DEVELOPMENT

(Piper and W. C. Foote, 1968).

Group	Treatment — Day of estrous cycle[1]: 4 + 5 to 22	Ewes with estradiol-induced CL (%)	Maintenance of natural and induced CL[3] — Days of treatment cycle: 22 (%)	37 (%)	Follicular development at day 14 of treatment cycle; ewes with at least one follicle 6 mm or > (%)
1	No injection	0[a2]	0[a]	0[a]	63[a]
2	2 mg	70[bc]	20[ab]	10[ab]	80[a]
3	2 mg + 0.5 mg	44[b]	56[bc]	20[ab]	0[b]
4	2 mg + 1.5 mg	100[c]	86[c]	40[bc]	12[b]
5	2 mg + 2.5 mg	86[c]	100[c]	100[c]	0[b]

[1] Levels used and duration of 17β-estradiol injections.
[2] Values not bearing the same superscript differ significantly (P<0.05).
[3] The patterns of regression were similar for natural and induced CL.

TABLE 3. INFLUENCE OF ESTRADIOL TREATMENT ON CORPUS LUTEUM MAINTENANCE AND FOLLICULAR ACTIVITY.[1]

(Piper and W. C. Foote, 1970)

Treatment Group	Ewes with CL maintained until autopsy — Natural (%)[2]	Induced (%)[2]	CL weight gm/CL	Ewes with follicles 3–6 mm at autopsy (%)[2]
C-10	100[a]	—	.638 ± .225[a2]	100[a]
C-15	80[ab]	—	.478 ± .023[a]	100[a]
T-10	100[ab]	[3]	.480± 0.56[a]	0[b]
T-15	100[ab]	100	.553 ± .277[a]	0[b]
T-22	50[b]	100	.524 ± .428[a]	0[b]
T-37	80[ab]	80	.485 ± 0.15[a]	20[b]

[1] CL which had regressed completely or contained no measurable progesterone were not considered maintained. No. of treatment group indicates the day of cycle autopsied.
[2] Values not bearing the same superscript letter differ significantly (P<0.05).
[3] Two of four ewes had luteinized follicles at day 10.

TABLE 4. EFFECTS OF ESTRADIOL TREATMENT ON CORPORA LUTEA AND OVARIAN VENOUS PROGESTERONE.[1]

(Piper and W. C. Foote, 1970).

Treatment group and day[2]	Total progesterone in CL μg/CL	Progesterone concentration μg/gm CL	Progestrone concentration μg/100 ml of plasma
C-10	39.2 ± 20.4[a]	34.8 ± 14.4[ab3]	116.7 ± 21.5[a]
C-15	12.6 ± 8.2[b]	12.9 ± 8.1[c]	37.6 ± 13.0[b]
T-10	17.6 ± 1.9[b]	20.1 ± 7.4[bc]	76.4 ± 21.8[ab]
T-15	13.6 ± 7.1[b]	20.3 ± 6.7[bc]	63.4 ± 25.0[ab]
T-22	15.8 ± 13.2[b]	30.3 ± 15.8[b]	78.1 ± 20.0[ab]
T-37	27.8 ± 7.6[ab]	46.3 ± 11.0[a]	32.8 ± 6.2[b]

[1] Corpora lutea in which progesterone could not be measured were excluded from the statistical analysis.
[2] C = control and T = estradiol treated; 10, 15, 22, 37 = day of experiment tissue was removed for analysis.
[3] $P<0.05$ for means not bearing the same superscript letter.

TABLE 5. INFLUENCE OF ESTRADIOL ON PITUITARY LUTEINIZING HORMONE AS DETERMINED BY THE OAAD METHOD OF BIOASSAY.[1]

(Piper and W. C. Foote, 1970)

Trial	Treatment group[2]	Ventral prostate wt. (mg)	Pituitary LH (μg/mg)
1	C-15		4.29 ± 3.69[a3]
	T-37		1.25 ± .98[b]
	C-10		.97 ± .55[b]
	T-15		.93 ± .69[b]
	T-22		.46 ± .56[b]
2	C-10	11.2 ± 1.7[a](186 ml)[4]	5.2 ± 2.1[a]
	T-10	18.6 ± 6.1[b](120 ml)	2.5 ± 0.5[a]

[1] LH activity estimated from ascorbic acid depletion by NIH-LH-S_6 standards as plotted on logarithmic paper.
[2] C = control and T = estradiol treated; 10, 15, 22, 37 = day of experiment tissue was removed for analysis.
[3] ($P<0.05$) for means not bearing the superscript letter.
[4] Values in parenthesis represents the equivalent amount of extracted plasma given during the 4-day injection period. Ventral prostate weights for 0, 22.2 and 44.4 μg NIH-LH-S_6 were 8.8, 17.7 and 19.7 mg respectively. Estimated levels of LH per ml plasma were .043 μg for the C-10 group and .336 μg for the T-10 group.

TABLE 6. EXPERIMENTAL DESIGN FOR EFFECT OF GONADAL AND GONADOTROPIC HORMONES ON CL FUNCTION IN SHEEP. (Dermody and W. C. Foote, 1969).

Treatment			Day CL removal		Total no. ewes
Hormone	mg	days[1]	16	26	
Control	–	–	4[4]	2[4]	13
Progesterone	5	8 to 25	5	1	12
Estradiol-17ß	1	8 to 25[2]	4	4	12
HCG	8[3]	8 to 25	4	4	13
Estradiol valerate	4	8 + 17	4	3	13
Testosterone	5	8 to 25	3	4	15
Norethandrolone	5	8 to 25	4	4	12

[1] Day 0 = day of estrus.
[2] Treatment given 2 × daily.
[3] 25 i.u./mg or 200 i.u./day.
[4] No. of ewes.

TABLE 7. MEAN EFFECT OF GONADAL AND GONADOTROPIC HORMONES ON CL WEIGHT AND GROSS MORPHOLOGY IN SHEEP. (Dermody and W. C. Foote, 1969).

Treatment	CL Weight		No. Ewes	% Ewes with	
	Day 16	Day 26[4]		Maintained CL[5]	Induced CL
Control[1]	781.5[a2,3] (4) ±68.0	781.5[a] (4) ±68.0	10	0[a]	0
Progesterone	506.1[bc] (5) ±64.9	—	11	18.2[ab]	0
Estradiol-17β	377.4[bd] (4) ±86.9	631.6[ab] (4) ±67.7	10	90.0[d]	16.6
HCG	639.6[e] (4) ±40.0	—	13	38.4[bc]	15.4
Estradiol	329.2[d] (4) ±104.5	356.6[c] (3) ±35.4	11	72.7[cd]	23.1
Testosterone	411.2[cdf] (3) ±14.4	519.1[b] (4) ±23.3	15	60.0[cd]	0.0
Noreth androlone	485.4[bf] (4) ±17.0	—	12	8.3[ab]	0.0

[1] Control CL could not be identified on day 26. Control CL information for day 16 used also for day 26.
[2] Numbers in () indicate number of ewes contributing to the mean.
[3] $P<0.05$ for means not bearing the same superscript letters.
[4] CL measured on day 26 were those formed at the time of estrus (day 0).
[5] CL maintained morphologically by gross observations on day 16.

TABLE 8. MEAN EFFECTS OF GONADAL AND GONADOTROPIC HORMONES ON CL PROGESTERONE CONTENT IN SHEEP. (Dermody and W. C. Foote, 1969).

	Day 16			Day 26[3]		
Treatment	No. Ewes	Total ug/CL	Conc. ug/gm	No. ewes	Total ug/CL	Conc. ug/gm
Control[1]	4	$15.1^{a2} \pm 3.0$	$18.95^{ab} \pm 2.9$	4	15.1 ± 3.0^{a}	$18.9^{a} \pm 2.9$
Progesterone	5	$12.9^{ab} \pm 2.5$	$25.9^{ab} \pm 3.5$	0	——	——
Estradiol-17β	4	$7.6^{c} \pm 1.1$	$23.7^{ab} \pm 4.3$	4	21.3 ± 3.4^{a}	$35.5^{b} \pm 6.1$
HCG	4	$8.9^{cd} \pm 2.3$	$15.1^{a} \pm 4.0$	0	——	——
Estradiol valerate	4	$6.5^{c} \pm 0.9$	$37.2^{c} \pm 19.0$	3	13.3 ± 5.8^{a}	$35.1^{b} \pm 12.2$
Testosterone	3	$11.1^{bd} \pm 2.1$	$27.3^{b} \pm 5.9$	4	16.8 ± 4.1^{a}	$32.0^{b} \pm 6.5$
Norethandrolone	4	$12.8^{ab} \pm 1.1$	$26.4^{ab} \pm 2.0$	0	——	——

[1] Control CL could not be identified on day 26. Control CL information for day 16 used also for day 26.
[2] $P<0.05$ for means not containing the same superscript letter.
[3] CL measured on day 26 were those formed at the time of estrus (day 0).

TABLE 9. EXPERIMENTAL DESIGN FOR EFFECTS OF ANTI-OVINE LH SERUM ON CL FUNCTION IN SHEEP.[1] (Dermody and W. C. Foote, 1969*b*.)

Trial	Treatment[2]	Day[3] treated	No. Ewes	Day of CL removal[4]
1. Estrous cycle	Control	None	6	11, 13, 15
	Serum	7, 9, 11	6	11, 13, 15
	Ab-LH serum	7, 9, 11	9	11, 13, 15
2. Hysterectomy	Control	None	3	42
	Ab-LH serum	37, 39, 41	2	42
3. Early Pregnancy	Control	None	2	36
	Ab-LH serum	30, 32, 34	2	36

[1] Day O = day of estrus.
[2] Serum was rabbit serum. Ab-LH serum was from rabbits containing antibodies developed to injected HIH-LHS13 at a titer of 1:500.
[3] Fifty ml of serum was injected intravenously on each day.
[4] CL were removed from an equal number of ewes at each day in trial 1.

TABLE 10. MEAN EFFECTS OF ANTI-OVINE LH SERUM ON CL WEIGHT IN SHEEP. (Dermody and W. C. Foote, 1969*b*.)

		No.	Day of CL Removal[3]		
Trial	Treatment	Ewes	11	13	15
1. Estrous cycle	Control	6	$811.3^{a1} \pm 256.6^{2}$	$528.9^{a} \pm 14.6$	$603.8^{a} \pm 27.3$
	Serum	6	$766.7^{a} \pm 77.7$	$494.7^{a} \pm 41.1$	$494.9^{b} \pm 30.3$
	Ad-LH serum	9	$464.5^{b} \pm 19.1$	$422.8^{a} \pm 75.0$	$383.9^{b} \pm 48.7$
			Day of CL Removal		
2. Hysterectomy			——	——	42
	Control	2	——	——	$590.1^{a} \pm 31.1$
	Ad-LH serum	2	——	——	$138.5^{b} \pm 18.1$
			Day of CL Removal		
3. Early pregnancy			——	——	36
	Control	2	——	——	$773.9^{a} \pm 8.8$
	Ab-LH	2	——	——	$596.9^{a} \pm 33.9$

[1] $P<0.05$ for means not bearing the same superscript letter.
[2] Standard error.
[3] CL were removed from equal numbers of ewes at each day in trial 1.

TABLE 11. THE EFFECT OF ANTI-OVINE LH SERUM ON TOTAL CL PROGESTERONE CONTENT IN SHEEP (UG/CL).
(Dermody and W. C. Foote, 1969*b*.)

Trial	Treatment[1]	No. Ewes	Day of CL removal[2]		
			11	13	15
1. Estrous cycle	Control	6	16.7 ± 5.8[b3]	14.4 ± 3.4[a]	10.3 ± 2.8[a]
	Serum	6	28.7 ± 6.2[a]	13.1 ± 3.4[a]	7.4 ± 2.2[a]
	Ab-LH serum	9	7.6 ± 2.2[c]	4.4 ± 1.4[b]	4.3 ± 1.9[b]
			——	——	42
2. Hysterectomy	Control	3	——	——	13.2[a] ± 1.75
	Ab-LH serum	2	——	——	<.2[b]
			——	——	36
3. Early pregnancy	Control	2	——	——	20.8[a] ± 5.95
	Ab-LH serum	2	——	——	16.2[a] ± 4.10

[1] Serum was rabbit serum. Ab-LH serum was from rabbits containing antibodies developed to injected NIH-LH-S_{13} at a titer of 1:500.
[2] CL were removed from equal numbers of ewes on each day in trial 1.
[3] $P<0.05$ for means not bearing the same superscript letter.

TABLE 12. THE EFFECT OF ANTI-OVINE LH SERUM ON CL PROGESTERONE CONCENTRATION IN SHEEP (UG/MG).
(Dermody and W. C. Foote, 1969*b*.)

Trial	Treatment[1]	No. Ewes	Day of CL removal[2]		
			11	13	15
1. Estrous cycle	Control	6	20.4 ± 0.5[a3]	27.3 ± 6.6[a]	17.1 ± 4.5[a]
	Serum	6	38.7 ± 12.0[b]	27.5 ± 8.1[a]	14.8 ± 3.9[a]
	Ab-LH serum	9	19.6 ± 5.0[a]	10.3 ± 3.4[b]	10.3 ± 3.5[a]
			——	——	42
2. Hysterectomy	Control	3	——	——	22.2[a] ± 1.72
	Ab-LH serum	2	——	——	<.2[b]
			——	——	36
3. Early pregnancy	Control	2	——	——	26.8[a] ± 7.4
	Ab-LH serum	2	——	——	26.5[a] ± 1.69

[1] Serum was rabbit serum. Ab-LH serum was from rabbits containing antibodies developed to injected NIH-LH-S_{13} at a titer of 1:500.
[2] CL were removed from equal numbers of ewes on each day in trial 1.
[3] $P<0.05$ for means not bearing the same superscript letter.

TABLE 13. EXCRETION OF ESTROGENS IN URINE AT 0, 7 AND 14 DAYS AFTER BREEDING (NG/MG URINARY CREATININE)[a]

		Days since breeding							
		Pregnant				Nonpregnant			
Study		0	7	14	Total	0	7	14	Total
		Montana[b]							
Cows	N	12				16			
	$\bar{x}$	59	56	56	57	104	98	76	110
	SE	8	7	5	2	15	18	8	8
	CV	44	45	30	39	58	74	43	62
	%[c]					176	175	136	163
Heifers	N	7				8			
	$\bar{x}$	50	46	44	47	110	76	79	88
	SE	8	7	8	4	66	16	33	12
	CV	40	41	45	40	169	61	118	105
	%[c]					220	213	173	198
Total	N	19				24			
	$\bar{x}$	55	52	52	53	106	91	77	91
	SE	6	5	4	3	23	13	12	10
	CV	44	43	36	41	108	72	75	91
	%[c]					192	175	148	172
		Indiana[d]							
Cows	N	12				23			
	$\bar{x}$	79	56	41	61	93	239	98	143
	SE	20	23	18	11	36	133	55	50
	CV	89	145	129	116	187	267	267	287
	%[c]					118	427	239	234
		Combined							
Total	N	31				47			
	$\bar{x}$	64	54	51	56[e]	99	163	87	117
	SE	8	9	7	5	21	66	27	25
	CV	75	96	80	83	146	276	215	250
	%[c]					155	302	171	209

[a] Montana data transformed from excretion for 24 hours/100 lb liveweight to ng/mg urinary creatinine using constant excretion of urinary creatinine of 0.9 mg/kg liveweight/hr.
[b] Acid hydrolysis of urine prior to extraction; paper chromatography followed by fluorometry for quantification and uncorrected for method losses.
[c] % change compared to cows conceiving.
[d] Two-step hydrolysis (enzyme-acid) for extraction, thin-layer chromatography, gas-liquid chromatography of the acetate derivatives and correction for method losses.
[e] Significantly lower than average of nonpregnant group ($P<0.01$).

TABLE 14. EXCRETION OF ESTROGENS IN URINE OF INDIANA COWS PREGNANT (P) AND NOT PREGNANT (NP) AFTER BREEDING.

Days after breeding	Cows	Total estrogen	Estrone	Estradiol- 17α	Estradiol- 17β
(Days)	*(No.)*	*(Ng/hr/kg/mg creat.)*	*(% of total)*		
Estrus	NP	79 ± 20	31	54	15
7	P	56 ± 23	18	59	23
14	P	41 ± 18	40	32	28
Estrus	23 NP	92 ± 36	39	36	25
7	23 NP	239 ± 133	17	23	60
14	23 NP	101 ± 54	64	26	10

TABLE 15. ESTIMATED MEAN LEVELS OF LH AT DIFFERENT STAGES OF GESTATION AND AT 20 DAYS POSTPARTUM.

(Svejda and W. C. Foote.)

Stage of gestation[1] (months)	Pituitary gland[2]						Jugular serum		
	OAAD Method		(ug/mg)	RIA Method		(ug/mg)	RIA Method		(ug/mg)
	No. Animals	Mean	Range	No. Animals	Mean	Method	No. animals	Mean	Range
1–2	3	10.93	6.14–16.76	4	8.55 15.5	3.7–	4	–	<FO.3–1.76
2–3	1	16.72	–	3	10.50	9.38–11.9	3	<0.3	–
3–4	2	24.43	24.37–24.50	4	16.03	8.09–28.6	4	–	<0.3–0.74
4–5	–	–	–	5	8.00	6.9–9.5	5	1.00	0.38–3.3
5–6	2	6.62	3.62–9.62	5	4.10	3.60 10.04	5	<0.3	–
6–7	–	–	–	–	–	–	–	–	–
7–8	1	12.16	–	4	6.62	5.3–9.16	4	–	<0.3–0.43
8+	–	–	–	1	0.35	–	1	<0.3	–
Post-partum (20 days)	9	10.3	6.96–15.88	9	10.47	4.5–21.1	–	–	–

[1] Animals varied in age and breeding. Stage of gestation for some of the animals was estimated from fetal crown-rump length. The 5 animals measured at 4–5 months of age were from U. S. Range Experiment Station at Miles City, Montana, and the 9 animals at 20 days postpartum were from University of Nevada at Reno.

[2] OAAD refers to Ovarian Ascorbic Acid Depletion method and RIA refers to Radio-Immunoassay method for LH analysis.

TABLE 16. MEAN VENTRAL PROSTATE WEIGHT OF IMMATURE HYPOPHYSECTOMIZED RATS INJECTED WITH SEPHADEX G-100 COLUMN FRACTIONS OF PHOSPHATE BUFFER EXTRACTS OF VARIOUS PLACENTAL TISSUE. (Lunnen and W. C. Foote, 1967.)

Exper. No.	Tissue fraction of hormone standard[1]		No. of rats	Ventral prostate wt. (mg)
1	Physiological saline		5	$7.4^{d2} \pm 1.30$
	NIH-LH-S_6	25 ug	5	$12.5^{b} \pm 1.04$
	NIH-LH S^b	50 ug	4	$22.6^{a} \pm 2.79$
	FC-1	20 mg	4	$9.6^{c} + 0.77$
	FC-2	10 mg	4	$9.7^{c} \pm 1.56$
	FC-3	20 mg	3	$12.1^{bc} \pm 1.05$
2	Physiological saline		8	$9.2^{b} \pm 0.56$
	FC-1	20 mg	8	$11.4^{a} \pm 1.52$
	FC-2	20 mg	4	$11.7^{a} \pm 1.63$
	FC-3	20 mg	5	$12.7^{a} \pm 1.47$
	FC-4	20 mg	3	$9.2^{b} \pm 0.58$
3	Physiological saline		11	$11.3^{e} \pm 1.95$
	NIH-LH-S_6	25 ug	7	$15.8^{bc} \pm 1.48$
	NIH-LH-S_6	50 ug	7	$21.9^{a} \pm 4.04$
	MIC-2	20 mg	6	$13.1^{de} \pm 1.08$
	MIC-3	20 mg	5	$14.7^{ed} \pm 1.45$
	MIC-4	20 mg	4	$16.9^{bc} \pm 2.25$
	MC-3	20 mg	7	$17.7^{b} \pm 0.83$
	MC-4	20 mg	3	$13.6^{bcd} \pm 0.51$
	FIC-1	20 mg	7	$14.0^{cd} \pm 2.45$
	FIC-2	20 mg	3	$14.4^{cd} \pm 2.62$
	FIC-4	20 mg	4	$10.9^{e} \pm 0.98$

[1] FC = fetal cotyledonary tissue; MIC = maternal intercotyledonary tissue MC = maternal cotyledonary tissue; FIC = fetal intercotyledonary tissue numbers represent respective column fractions.
[2] $P<0.05$ for means not bearing the same superscript letter.

TABLE 17. MEAN VENTRAL PROSTATE WEIGHTS OF IMMATURE HYPOPHYSECTOMIZED RATS INJECTED WITH COLUMN FRACTIONS OF PHOSPHATE BUFFER EXTRACTS OF REPRODUCTIVE TISSUES AND OF MATERNAL JUGULAR AND UTERINE SERUM AND OF FETAL SERUM. (Lunnen and W. C. Foote, 1967.)

Tissue fraction or hormone standard		No. of rats	Ventral prostate wt (mg)
Physiological saline		5	$8.0^{e1} \pm 1.22$
NIH-LH-S_6	25 ug	6	$13.7^{b} \pm 3.11$
Jugular serum fraction 1	20 mg	5	$11.1^{cd} \pm 1.02$
Jugular serum fraction 2	20 mg	7	$9.8^{cde} \pm 2.01$
Uterine serum fraction 1	20 mg	4	$8.8^{de} \pm 2.14$
Uterine serum fraction 2	20 mg	7	$11.4^{c} \pm 0.80$
Fetal serum fraction 1	20 mg	6	$11.3^{cd} \pm 1.83$
Fetal serum fraction 2	20 mg	7	$12.2^{bc} \pm 1.00$
MC-2[2]	20 mg	6	$13.5^{b} \pm 1.81$
MIC-1	20 mg	3	$16.5^{a} \pm 1.32$

[1] $P<0.05$ for means not bearing same superscript letter.
[2] MC = maternal cotyledonary and MIC = material intercotyledonary fractions. The numbers represent respective column fractions.

TABLE 18. AVERAGES AND STANDARD DEVIATIONS IN DAYS OF POSTPARTUM INTERVALS IN TREATED AND UNTREATED BEEF COWS (10 ANIMALS PER GROUP; D. Foote.)

Intervals from parturition	Treatment groups			
	Untreated	P5–15, P17[a]	P14–24, E26[b]	P23–33, E35[c]
Uterine involution				
Av.	43[d]	40[d]	41[d]	41[d]
S.D.	9.0	9.2	6.4	6.0
First estrus[f]				
Av.	53	22	29	43
S.D.	20.5	10.6	2.9	14.5
First ovulation				
Av.	50[d]	30[e]	38[d,e]	41[d,e]
S.D.	21.7	14.5	11.9	12.2
Conception				
Av.	87[e]	56[d]	48[d]	66[d,e]
S.D.	42.8	22.4	21.7	22.9

[a] 50 mg. progesterone/cow daily day 5 through 15 postpartum, 10 mg. estradiol-17β day 17.
[b] 50 mg. progesterone/cow daily day 14 through 24 postpartum, 10 mg. estradiol-17β day 26.
[c] 50 mg. progesterone/cow daily day 23 through 33 postpartum, 10 mg. estradiol-17β day 35.
[d,e] Averages on the same line not having the same superscript differ significantly ($P<0.05$).
[f] Averages for first estrus differ significantly ($P<0.05$) among groups. However, comparisons of combinations of means were not made because of heterogeneity of variance.

TABLE 19. AVERAGES AND STANDARD DEVIATIONS, IN DAYS, OF POSTPARTUM INTERVALS IN DAIRY COWS. (D. Foote.)

Postpartum intervals	Untreated	Treated
Uterine involution		
Av.	47.65	43.62
S.D.	10.38	8.93
First ovulation		
Av.	28.98	19.58
S.D.	12.94	4.33
First estrus		
Av.	39.44	26.60
S.D.	16.40	13.52
First to second ovulation		
Av.	20.98	21.25
S.D.	5.68	5.47

TABLE 20. AVERAGE PITUITARY AND CORPUS LUTEUM ACTIVITIES AT DIFFERENT REPRODUCTIVE STAGES. (D. Foote.)

		Pituitary			Corpus Luteum				
Day of slaughter	No. of animals	LH concentrations ug/mg.[a]	FSH concentrations ug/mg.[a]	LH:FSH ratio	Av. C L wt. (gm.)	Av. progesterone ug.	Av. progesterone ug./gm.	Av. 20 B ug.	Av. 20 B ug./gm.
B + 275[b]	5	5.0	4.4	1.1	4.1	28.5	7.9	17.9	4.4
P + 0[c]	5	2.8	4.5	0.6	2.8	1.6	0.6	1.1	0.4
P +20[c]	4	7.5	. .	0.0	0.0	0.0	0.0	0.0	0.0
E +0[d]	5	6.4	<1.2	5.3	0.0	0.0	0.0	0.0	0.0
E + 1[d]	4	3.0	<1.2	2.5	0.7	3.4	7.3	2.6	6.6
E + 16[d]	5	18.3	1.4	13.1	5.3	82.3	15.0	42.0	7.7
E + 19[d]	4	16.2	1.5	10.8	3.2	25.5	5.7	10.5	2.3

[a] Expressed as ug. equivalents of NIH-FSH-S_1 per mg. dry weight of anterior pituitary gland.
[b] 275th day of gestation.
[c] Days after parturition.
[d] Day of postpartum estrual cycle. Day 0 is day of estrus.

TABLE 21. LH ACTIVITY[a] OF UNOPERATED AND OPERATED BEEF COWS POSTPARTUM. (D. Foote).

Treatment		LH (ug./mg dry pituitary) OAAD Assay	Radio Immuno Assay
Intact	$\overline{X}$	10.4	9.4
	SD	3.19	1.03
Ovariectomized[b]	$\overline{X}$	6.5	6.5
	SD	2.89	.41
Ovariectomized +	$\overline{X}$	5.8	5.6
Hysterectomized[b]	SD	.62	2.35

[a] LH levels 20 days after calving.
[b] All operations were performed within 24 hours after calving.

TABLE 22. STEROID SYNTHESIS (MuM PER FLASK) IN ADRENAL HOMOGENATES INCUBATED WITH 200 MuM PROGESTERONE-4-^{14}C OR PREGNENOLONE-4-^{14}C.

Substrate	Metabolite	Tissue eq. and time	"Low" Jersey*	"Normal" Jersey*	Holstein*
Pregnenolone	Progesterone	20 mg – 5 min	84.5 (3)	61.8 (7)	60.3 (18)
		25 mg – 15 min	169.5 (4)	183.0 (2)	179.6 (2)
Progesterone	17α-hydroxy-progesterone	25 mg – 15 min	2.6 (4)	6.2 (2)	8.8 (2)
		40 mg – 10 min	13.8[a] (3)	21.6 (7)	19.6[b] (19)
		100 mg – 10 min	—	15.2 (6)	14.6 (15)
		200 mg – 30 min	17.5 (4)	17.4 (8)	12.3 (20)
Progesterone	Deoxycorti-costerone	25 mg – 15 min	7.6 (7)	4.2 (4)	5.0 (3)
		40 mg – 10 min	10.8 (3)	13.2 (7)	11.6 (19)
		100 mg – 10 min	—	28.8[a] (6)	19.9[b] (15)
Progesterone	Cortico-sterone	40 mg – 10 min	5.9[a] (2)	8.9[b] (5)	9.8[b] (17)
		100 mg – 10 min	—	20.4 (6)	25.5 (15)
		200 mg – 30 min	76.6[a] (2)	53.2[b] (7)	63.3 (17)
Progesterone	11-deoxy-cortisol	25 mg – 15 min	2.0 (7)	1.6 (4)	2.3 (3)
		40 mg – 10 min	3.6[a] (2)	8.8[b] (5)	7.8[b] (17)
		100 mg – 10 min	—	37.0 (6)	24.9 (15)
		200 mg – 30 min	22.1[a] (2)	17.4 (7)	9.1[b] (17)
Progesterone	Cortisol + cortisone	25 mg – 15 min	1.2[a] (2)	1.2 (4)	1.4 (3)
		40 mg – 10 min	0.8 (4)	0.6 (8)	1.3 (20)
		100 mg – 10 min	—	7.2[a] (6)	13.2[b] (20)
		200 mg – 30 min	67.3[a] (4)	86.4 (8)	87.8[b] (20)

* Numbers in parentheses represent the number of animals in the reported average; a and b: means with different superscripts are significantly different at the 0.05 level.

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